

ON THE PRESENCE OF CYSTS IN THE HUMAN PITUITARY¹

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SEVENTEEN FIGURES

INTRODUCTION

Occasional cysts have been described in the pituitaries of amphibians (Copeland, '43 and Kent, '45), reptiles (Altland, '39 and Poris, '41) and birds (Lothringer, 1886; Collin, '26 and Rahn, '39).

Pituitary cysts have been described in a wide variety of mammals; marsupial "Gamba," *Didelphys aurita* (Martins, '33), guinea pig (Vanderburgh, '17 and Chadwick, '37), rat (Martins, '33 and Oppen, '40), tiger (Hanstrom, '46), badger, ferret and polecat (Hanstrom, '47), cat (Herring, '08) and dog (Bell, '19, Severinghaus, '38), *Cercopithecus* and the sacred baboon, *Papio hamadryas* (Hanstrom, '48).

Cysts in the human pituitary are rarely described unless they attain considerable size and are of clinical significance. Some of the papers on pituitary cysts in man derived from Rathke's cleft or residual lumen, are: Erdheim ('04), Cushing ('12), Duffy ('20), Kiyono ('26), Frazier and Alpers ('34), Gillman ('40) and Hanstrom ('48).

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It is the purpose of this paper to describe the incidence and histological appearance of cysts originating from Rathke's cleft in the human pituitary and to discuss the relationship of these cysts in man and the lower animals.

OBSERVATIONS

Classification: Frazier and Alpers ('34) consider the term craniopharyngioma a misnomer and misleading and state (p. 984) that it should be discarded in favor of tumors of the hypophyseal stalk; furthermore they recommend that the so-called tumor of Rathke's pouch should be designated tumor of Rathke's cleft. We agree with Frazier and Alpers that there is histologic justification for the separation of Rathke's cleft tumors from those of the stalk. The hypophyseal vesicle or sac, a later stage of Rathke's pouch, is composed of stratified columnar epithelium. From this tissue develops the anterior lobe, but a single layer of cylindrical epithelium persists in the adult gland lining the cleft or Rathke's cleft (Duffy, '20, Frazier and Alpers, '34, fig. 1). In general the epithelium of the stalk tumors is cuboidal or stratified squamous, frequently with intercellular bridges and that of Rathke's cleft tumors or cysts is cuboidal or stratified columnar ciliated.

Most of the cysts described by us were small sacs, situated primarily in the epithelial parts of the gland. They were provided with a capsule and contained semifluid material. Their epithelium varied from low cuboidal to stratified columnar ciliated. Some of the sac-like structures communicated with the cleft or follicles in pars intermedia. These might be considered as diverticula, however, both the completely closed and the communicating sacs were similar histologically. For the sake of simplicity both are referred to as cysts.

Eleven of the cysts were derivatives of Rathke's cleft. Three of them (M100, M217 and M218) were classified as cystic clefts (one complete and two partial). The majority (M92, M98, M101, M172, M183, M230 and M246) were included in the category of simple and compound follicular cysts. The cysts in M204 were classified as multiple follicular cysts.

The cyst in M248 was classified as a multilocular cyst. This is a degenerative process and this cyst is similar to multilocular cysts of the ovary and other organs. The remaining cyst (M115) was a persistent canal and belongs to the stalk cysts or tumors.

Incidence. Cysts were observed in 13 (13%) of the 100 pituitaries in our series (M92 male aged 65 years, M98 female aged 80, M100 male aged 65, M101 male aged 37, M115 male aged 40, M172 female aged 83, M183 female aged 39, M204 male aged 40, M217 male aged 40, M218 female aged 17, M230 male aged 35, M246 male aged 20 and M248 female 3.5 years). For the cause of death and other histological findings the reader is referred to the paper by Shanklin ('48a, table 1).

The youngest pituitary with a cyst was from a 3.5-year old girl and the oldest from an 83-year old woman. Of the total 100 pituitaries 47 were from individuals varying from the newborn to 19 years, 16 from persons 20 to 39 years and 37 between 40 and 86 years. Cysts were only found two times (4.3%) in the younger age group (newborn to 19 years), 4 times (25.0%) in the middle group (20 to 39 years) and 7 times (18.9%) in the older group (40 to 86 years). Cysts were found in 5 of the 36 female and in 8 of the 64 male pituitaries in the series.

Location, shape and size. The persistent canal in pituitary M115 was tubular and consisted of two parts, a smaller round lumen ($.07 \times .07$ mm) (fig. 12) located immediately beneath the capsule of the upper surface of the pars anterior and a part with a wider lumen ($.26 \times .29$ mm) situated in the capsule itself (fig. 13). The total length of the canal was .30 mm.

We are referring to three of the cysts as cystic clefts because they represent abnormally large clefts. These did not penetrate into the substance of the adjacent part of the pars intermedia nor pars anterior. One of these (M218) retained the fetal condition and the greatly enlarged cleft (fig. 4) extended across the entire width of the pituitary thereby separating a greatly reduced pars intermedia from the pars anterior. This cystic cleft reached its maximum antero-poste-

rior extent along the midline (2.56 mm). Even at the outer side as it approached the capsule the cleft was wide (1.50 mm on one side and 1.75 mm on the other). The cyst in M100 was a distension of the cleft in the upper half of the pituitary. On cross section this cyst measured $.52 \times 1.61$ mm. The cyst in M217 caused distension of the lower third of the cleft. It was bounded below by the capsule, posteriorly the nervosa was lined by a few cells that represented the pars intermedia, anteriorly it bulged into the pars anterior. This cyst was $.89 \times 4.82$ mm on cross section. These two were classified as partial cystic clefts.

Most of the simple follicular cysts described below end blindly in the pars anterior and extend proximally to communicate with follicles in the region of the pars intermedia.

The cyst in M92 was a macroscopic cyst located in the distal part of the pars anterior (fig. 1). In sections where it reached its maximum diameter it was bounded on one side by the pituitary capsule and on the other side by the pars anterior. Caudally it communicated with enlarged follicles in pars intermedia. Its maximum diameter on cross section was 2.5×2.9 mm.

The cyst in M98 was situated near the midline (fig. 2) of the pars anterior and extended anteriorly almost to the capsule. It was roughly oval in shape (2.00×3.02 mm) with the long axis parallel to the capsule. Proximally it opened into follicles in the pars intermedia.

Pituitary M101 had a small, but well defined cyst (fig. 7), located in the proximal and superior part of the pars anterior. Nearby were several smaller cysts, all of which communicated with one another. The maximum size of the largest cyst on transverse section was $.17 \times .23$ mm. The largest cyst was relatively round on the inner side but somewhat flattened on the outer side. Proximally this cyst communicated with the pars intermedia follicles.

The macroscopic cyst in M172 was situated near the midline of the pars anterior. The most anterior part of this cyst was bounded by the pituitary capsule. Its maximum size in

the transverse plane was 1.41×2.40 mm. Distally it communicated with an occasional small cyst and proximally it opened into greatly enlarged follicles in the pars intermedia area.

The relatively large cyst in M183 was chiefly in the inferior and proximal part of the pars anterior (fig. 14). The anterior and superior parts were bounded by the pars anterior, the inferior by the pituitary capsule and the posterior part by the pars nervosa and a few cells representing the pars intermedia. On cross section this cyst was 1.31×1.64 mm. This cyst extended laterally parallel to the anterior surface of the pars nervosa and on one side extended beyond the lateral limit of pars nervosa between the capsule and the pars anterior.

Unlike most of the others, pituitary M204 was sectioned in the horizontal plane (fig. 3). It contained a number of large cysts situated between the pars intermedia and pars anterior. These were classified as multiple follicular cysts. Actually the pars intermedia was so greatly reduced that these cysts were really bounded by the pars nervosa. They form a series of cysts surrounding a considerable part of the anterior surface of pars nervosa. There was also a bilateral cyst on each of the posterior aspects of the pars anterior. These were bounded anteriorly and laterally by the pars anterior, posteriorly by a very thin bulging capsule and medially by the pars nervosa. Antero-medially they communicated with the cysts surrounding the anterior surface of the pars nervosa. The cyst measured (fig. 3) had a large oval portion occupying one of the posterior poles of pars anterior and a broad stem that projected anteriorly between the lateral aspect of the pars nervosa and the medial side of pars anterior. On cross section the main part of this cyst was 1.75×2.86 mm and the stem $.59 \times 2.25$ mm.

Pituitary M230 had two macroscopic cysts. One (fig. 5) was located in the posterior and superior area of the pars anterior and the other in the corresponding position of the inferior side. The one in the upper position was the smaller.

It communicated inferiorly with the follicles of pars intermedia. The cyst in the inferior position communicated throughout its extent with large cystic follicles in the pars intermedia. The largest cyst in this specimen measured on cross section 1.10×1.50 mm.

The cyst in M246 was one of the smallest observed. It was located in the inferior and proximal part of pars anterior and communicated posteriorly with enlarged follicles in the pars intermedia. It measured on cross section $.37 \times .39$ mm.

The very large macroscopic cyst in M248 was classified as multilocular because it opened into the many smaller cystic follicles that surround it (fig. 6). In outline the cyst was very irregular because of the numerous communications with the smaller cysts all of which directly or indirectly opened into the major cyst.

This cyst was located near the center of the pars anterior. Unlike most of the other cysts it did not reach the capsule of the pituitary. From its central location it extended superiorly and proximally. One thin extension reached the upper part of the pars intermedia where it communicated with follicles. The part of the cyst included in figure 6 was $.72 \times 1.27$ mm.

Histological appearance. These cysts were all surrounded by capsules, consisting chiefly of collagenous fibers. The thickness of the capsule, however, was extremely variable. The collagenous nature of most of these fibers was confirmed by Mallory stain. The two exceptions, discussed below, were specimens M183 and M218.

The capsules of the simple and compound follicular cysts are described first. The capsules around the cysts in M172 and M230 were poorly developed and only consisted of scattered fibers. The cyst in pituitary M294 had a thin medium capsule that nearly surrounded the cyst. Specimens M92 and M98 had cysts with thin capsules on the proximal side which were fairly thick on the distal side. The cyst in pituitary M101 was nearly surrounded by a thick capsule (figs. 7 and 8) which consisted of densely packed fibers. The trabeculae in

the pars anterior of this specimen were highly developed and the outermost fibers of the cyst capsule were continuous with those of the trabeculae. Specimen M115 had a very thick capsule which completely surrounded the cyst (figs. 12 and 13). There was a very thick capsule around the main cyst in pituitary M183 and thin, but well developed, capsules around the nearby smaller cysts. The capsule of this cyst in hematoxylin eosin preparations showed hyalinization and degeneration. In the Mallory preparations the capsules of both the large and the smaller cysts were stained an intense red. The colloid material in the cysts in the same sections, was stained dark blue; the same was true of the collagenous fibers in the pituitary capsule. The cyst in M246 had a thin but compact and continuous capsule on the distal side, but thick scattered masses of fibers on the proximal side.

The capsule around the cystic cleft in M100 consisted of scattered fibers only, but that of M217 was moderately thick and surrounded the entire cleft. The capsules of both stained blue with Mallory. The anterior capsule of the cystic cleft in M218 varied from thin to very thick, but the capsule of the posterior side was thick throughout. Many of the nuclei in this capsule were large, long and fusiform and appeared more like the nuclei of smooth muscle than of fibroblasts. Both the colloid and the capsule of this cyst were stained bright red in the Mallory preparations.

The capsule of the multilocular cyst in M248 was well defined and stained deep blue in the Mallory preparations in a few places, but was lacking in many other places. Part of the capsule, which stained red after Mallory, had large fusiform nuclei resembling those of smooth muscle.

Cyst contents. Most of the cysts (M100, M172, M183, M217, M218, M230, M246 and M248) were filled with a homogeneous colloid material which was usually stained blue in the Mallory preparations. Two of the cysts (M92 and M115) had granular material (figs. 13 and 15) which appeared to be a protein precipitate. The small cyst in pituitary M101 was filled with granular material (fig. 7) which stained positive with mucic-

carmine. The obvious sources of the mucus secretion were the large mucous cells among the ciliated lining cells. The contents of the cyst in specimen M204 were clearly of two varieties. Most of the material was homogeneous and colloid-like. In the region immediately above large mucous cells, however, there were areas of granular material stained intensely blue in Mallory preparations (fig. 17) and lighter in color after hematoxylin and eosin (fig. 10). Mucicarmine proved this material to be mucus. Most of the material in the cyst in M98 was stained bluish-purple after Mallory, but sharply separated large oval areas of brownish-orange colored material was scattered throughout the cyst. Both areas were filled with fairly large vacuoles (fig. 9), which probably contained neutral fat. There was no evidence of cholesterol deposits. On the basis of differential staining and the presence of vacuoles this cyst appeared to contain three different kinds of material.

Lining cells. The lining cells varied greatly within the same cyst, and those of the different cysts were markedly different. For this reason the lining cells of most of the cysts are described separately.

The cyst in pituitary M92 had a single layer of low cuboidal to tall columnar epithelium ($10.5\ \mu$), with cilia $4.0\ \mu$ high, lining the posterior wall but there were no cells on the anterior wall. No goblet cells were observed in this cyst.

The lining cells of the cyst in M98 varied from flat to stratified columnar epithelium with beautiful cilia. The columnar cells were $11.4\ \mu$ high and the cilia $3.4\ \mu$. The nuclei were large and had well stained fairly large chromatin granules. No goblets cells were observed.

Most of the lining cells in the distal part of the cyst in specimen M100 were missing, but proximally there were cuboidal cells. Along the inner curvature of the cyst, where it communicated with cysts in the region of the pars intermedia, some of the lining cells had lightly stained basophilic granules.

The lining cells of the smaller cysts in M101 varied from flat to cuboidal epithelium. The wall of the distal part of

the largest cyst was also lined by cuboidal cells, but proximally the cuboidal cells were replaced by simple and then stratified columnar ciliated (fig. 7). In the latter there were two layers of nuclei which, except for shape, were essentially similar to one another (fig. 8). The deeper layer of nuclei were more oval with their long axes parallel to the well developed cyst capsule. The upper nuclei were round or slightly oval. The nuclear membranes were fairly thick and darkly stained, the chromatin granules were coarse and scattered. Nucleoli were not observed. Another variety of nucleus was located at the base of large oval mucus-secreting cells. The very darkly stained nuclei of these cells were flattened against the basement membrane. In the Mallory preparations the mucus-secreting cells had bluish-purple threads of granular material. From the free surface of the cells similar granules extended up into the adjacent homogeneous material of the cyst, and stand out in sharp contrast to the red stained colloid. In places these mucus-secreting cells have the typical goblet shape (figs. 7 and 8).

Some of the cells appeared to be mucus-secreting cells after the secretory phase. They had small elongated nuclei and scanty cytoplasm which was more darkly stained than that of the surrounding cells. Overlying the area of ciliated cells, in hematoxylin eosin stained sections, was a pinkish stained darker area (figs. 7 and 8), the nature of which was not determined.

The lining of the larger part of the persistent canal in pituitary M115 was a single layer of flat cells with clear cytoplasm (fig. 13). Toward the pituitary capsule an occasional larger cell appeared. More distally there were from two to 4 layers of round to cuboidal cells with abundant cytoplasm (fig. 12). The deeper nuclei were slightly flattened while the upper ones were oval or round. The chromatin granules were fairly large. A few cells with darkly stained small irregular shaped nuclei were also present. These cells had scanty cytoplasm and were clearly intrusive elements. No mucous cells nor cilia were observed.

The cysts in pituitaries M182 and M183 had very few lining cells and in many places none at all. Where present they formed one to two layers with no special features.

Many variations were shown in the cells lining the numerous cysts in M204. The distended part of the cysts toward the pituitary capsule was usually devoid of lining cells. On the medial surface of one of the cysts there was an area of tall (19.5μ) simple columnar cells in which groups of cells with abundant long cilia (8.0μ) alternated with large goblet cells (fig. 10). The nuclei of the ciliated cells were elongated with their long axes parallel with that of the cell. The nuclei of many of the goblet cells were flattened and located near the basement membrane. In Mallory preparations the mucus-secreting cells were seen filled with bluish stained granules which were fairly evenly distributed in the cell and the overlying area.

In some areas of the largest cyst in M204, where both goblet and ciliated cells were lacking, the cells were approximately cuboidal. Immediately above the free border of these cells there were many round clear vacuoles, similar to those above the lining cells of thyroid follicles, in the relatively homogeneous colloid. Lying along the nervosal side of one of these cysts was an area of tubular glands. These glands had a single main duct which opened into the cyst cavity (fig. 11). Another cyst protruded deep into the distal part of the pars nervosa at the junction of the stalk and the infundibular process. Nearly all the original lining cells of this cyst were lacking but more than half of its proximal wall was formed by a large area of tumorette cells (fig. 16).

The cystic cleft in M217 was lined by cells varying from simple cuboidal to low stratified columnar ciliated. A few, lining the anterior surface, were typical basophils. No goblet cells were seen.

The lining cells of the anterior and posterior walls of the large cystic cleft in specimen M218 varied in their morphology. In most places the lining cells of the posterior wall were missing; a few areas had low rectangular cells with flat

darkly stained nuclei. Several small areas were lined by simple to stratified columnar lining cells with very tall cilia. Most of the anterior surface was lined by a thick layer of stratified columnar epithelium. Among these were tall beautifully ciliated cells alternating with large mucus-secreting cells.

The large cyst in pituitary M230 lacked lining cells in most places. Part of the cyst wall was lined by flat to rectangular cells with flat darkly stained nuclei and with clear cytoplasm. These resembled the cells lining the posterior surface of the cornea. Several small nearby cysts were lined by cuboidal cells.

The cells lining the cysts in M246 varied from cuboidal to stratified columnar. A few areas of the latter were ciliated. Some basophils were seen among the lining cells but there were no goblet cells.

The lining cells in the multilocular cysts in pituitary M248 were very variable. Considerable areas of the main cyst were devoid of lining cells, and those present varied from low rectangular to cuboidal. In some places there were small areas of low stratified columnar cells. Some of the nuclei were stained normally, but many others were darkly stained and pyknotic. The latter were surrounded by eosinophilic cytoplasm. The smaller cysts were lined by flat to cuboidal cells with relatively lightly stained cytoplasm. Many well stained basophils and eosinophils were seen in the nearby areas but not in the cysts themselves. In general there was a decrease in the height of the epithelium in passing from the more distal to the large central cyst. No cilia nor goblet cells were observed.

DISCUSSION

Incidence. In the salamander, *Triturus viridescens*, Cope-land ('43) found cysts in 5 out of 300 pituitaries, and Kent ('45) found cysts in two out of 260 specimens. Altland ('39) found only one cyst in 200 pituitaries of the fence lizard, *Sceloporus undulatus*. These cysts have a high incidence

in the chick for Rahn ('39) observed one or more cysts in nearly every chick pituitary examined. Pituitary cysts have been described in a number of mammals but very little data is available on their incidence. Chadwick ('37) found vesicles in the anterior lobe, which were lined with ciliated columnar epithelium, in 10% of the guinea pig pituitaries studied and Oppel ('40) found 14 rat pituitaries with cysts among the 139 specimens examined.

Cysts were found in 13 of the 100 human pituitaries included in our series (36 females and 64 males). Cysts occur in about the same proportion in the two sexes (12.5% females and 13.9% males) and most frequently in the middle age group. Cushing ('12) included (fig. 271) a section of a pituitary with a greatly enlarged cystic cleft from a 10-month old boy with pituitary deformity resulting from pressure caused by congenital hydrocephalus. The only data available on the occurrence of cysts in young animals are that of Rahn ('39) who found ciliated cysts in the chick pituitary on the 14th day of incubation and non-ciliated colloid filled cysts even earlier.

Location. Ten of the cysts in our series were located chiefly in the pars anterior, but communicated with follicles in, or near, the pars intermedia. The cystic canal observed in M115 was confined entirely to the parts anterior with one end penetrating into the capsule of the upper surface. The remaining three classified as cystic clefts were located between the pars anterior and pars intermedia. One of the very large cysts in specimen M204 extended for a considerable distance into the nearby infundibular process.

Although the cysts described in the amphibians, reptiles, birds and mammals were usually associated with pars anterior, the 5 observed by Copeland ('43) were in the pars intermedia. Kent ('45) found cysts in two *Triturus* pituitaries between the pars intermedia and anterior.

Histological appearance. All of the cysts in our series were surrounded by capsules. In some the capsule consisted of scattered collagenous fibers; one had a capsule that was

incomplete, another had a thick densely packed capsule of uniform thickness completely surrounding the cyst. Several cysts had well developed capsules which were thin on the proximal and thick on the distal side. The thick capsule of one cyst was hyalinized and failed to give the typical reaction after staining by the Mallory method. This is not surprising for old collagen often fails to take the normal stain. The multilocular cysts lacked capsules around the smaller cysts.

The most striking characteristic of most of these cysts in man was the presence of ciliated columnar epithelium. Copeland ('43) found ciliated cysts in the pituitaries of *Triturus* but did not describe their histology, Kent ('45) also saw several similar cysts in this animal and says they were lined by low ciliated epithelium. Altland ('39) records the presence of a ciliated cyst in a lizard, but does not describe the epithelium. The cyst in *Anolis carolinensis* described by Poris ('41) was lined chiefly by the normal glandular cells of the pars anterior, mostly basophils, and a restricted area of ciliated cuboidal epithelium. Collin ('26) says that the lining cells of cysts in the chick pituitary frequently formed an incomplete layer, the ciliated cells were of variable height and in places tall and cylindrical. Rahn found cells which varied from simple squamous to simple cuboidal with long cilia and basal granules in the chick.

Vanderburgh ('17) said that in the guinea pig the so-called colloid vesicles of the pars intermedia and occasionally of the pars anterior were usually lined with ciliated epithelium. Chadwick ('37) found ciliated columnar epithelium lining the pars anterior vesicles of the guinea pig. Martins ('33, fig. 1) found cuboidal cells with cilia 5μ long lining the cyst in one rat, and simple columnar cells with cilia of 5μ in another. Of the 16 cysts described by Oppen ('40) in the anterior lobe of the rat 14 were lined by cuboidal to columnar ciliated epithelium. In only three rats were the cilia continuous around the cyst wall; in most cysts areas of non-ciliated cells interspersed with ciliated were present. One of the large double colloid cysts described in the pars anterior of

the tiger by Hanstrom ('46, figs. 6 and 7) was lined partly by ciliated epithelium. Severinghaus ('38) said that the cysts in the anterior lobe of the dog were frequently lined with ciliated columnar epithelium, but they might be lined by normal acidophils, basophils and chromosomes, some of the latter being ciliated.

Ciliated cells have been described in man lining the cleft and also in cysts. Bryant ('16) reported ciliated epithelium as a constant finding in the human pituitary, but this frequency has not been confirmed. Kiyono ('26) found ciliated epithelium in one pituitary out of 50 and that one was from a premature infant. Rasmussen ('29, figs. 1 to 5) found ciliated epithelium in the residual lumen and in a small cyst of the anterior lobe in a 28-year old male, and lining the residual lumen of a 71-year old male. These ciliated cells were mostly found on the anterior side of the lumen. Gillman ('40) described ciliated and mucous cells in 16 out of 80 Bantu pituitaries and in the pituitary of a baboon. Several of the Bantu pituitaries contained cysts. One normal specimen had several cysts in the anterior lobe (fig. 1), and one acromegalic pituitary had numerous widely separated cysts in the anterior lobe (fig. 2). The cysts in both of these were partially lined by ciliated and mucous cells. Gillman always found ciliated cells associated with mucus elements, but the latter might occur alone.

The writer (Shanklin, '48b) frequently found follicles in the human anterior lobe containing concretions. These follicles were lined by the normal anterior lobe parenchymatous cells, none of which were ciliated.

The nature of the cyst contents has never been accurately determined. Kent ('45) found the lumen in *Triturus* filled with detritus consisting chiefly of cell remnants. Poris ('41) found in *Sceloporus* a coagulum with concentric lines which suggested concretions. The coagulum was separated from the lining cells by a zone of lighter blue material containing small deep blue spheres. Some of the latter were found deep in the cyst and appeared to be adding to the cyst contents.

Altland ('39) said there was no evidence that the cysts described in the fence lizard were concerned with the production of secretory material. Collin ('26) found in the chick a reticulated or granular coagulum which gave a typical mucus-staining reaction. Rahn ('39) also found actively secreting mucous cells in the chick pituitary.

Opper ('40) said that Mayer's mucicarmin stain proved that the homogeneous material which filled the cysts in the rat was mucinous. Gillman ('40) found in the cavities of these cysts in the human detritus, colloid and mucus. Some of the cyst cavities in our series were filled with a homogeneous colloid material. Hanstrom ('47, fig. 15) described large concretions in a cyst in the pars intermedia of the badger. Shanklin ('46a and '48a) described in the human pituitary concretions derived from mesothelium. None of the material of the cyst cavities of our series showed a tendency toward stratification. Those cysts in our material having goblet cells among the lining cells always contained varying amounts of mucinous material.

One of the cysts in our series communicated by a duct with an area of glands of Erdheim ('04). These are tubular glands located in the proximal part of the pars nervosa. Lewis and Lee ('27) published an excellent description of the morphology of these glands and Guizzetti ('26) made a thorough study of their incidence and histology in man. Erdheim referred to them as salivary glands, but Guizzetti says (p. 731) that they are specific sero-albuminous glands, which are different from the salivary glands.

Other possible secretory cells associated with one of the cysts in pituitary M204 were the tumorette cells. Shanklin ('47) found that these tumorettes were derived from two varieties of cells; those lining follicles in the pars intermedia, and by transitional stages from areas of typical basophil cells located in the infundibular process. Both of these varieties have a secretory function and the author suggested that these tumorette cells likewise had a secretory function.

Some acidophils, basophils and chromophobes have been described among the cells lining pituitary cysts. Poris found normal glandular cells of the anterior lobe, chiefly basophils and a few acidophils, lining the cyst in a reptile. Practically no chromophils nor chromophobes were observed in the cysts in our series except in the cystic clefts. In these they were limited to the anterior wall of the cleft. Gillman found some basophils lining some of the cysts in an acromegalic pituitary.

The deep blue spheres observed in the lumen by Poris, the marginal vacuoles described by Hanstrom ('48, figs. 2 and 3) in *Hapale jacchus* and the vacuoles observed in one of our specimens (M204) are all evidence of secretory activity.

The evidence for secretory products entering these cysts from several varieties of cells is well established. Apparently in most cases the rate of absorption from the cysts is approximately equal to the rate of accumulation, therefore, these cysts seldom become of clinical importance.

Embryological interpretation. The simplest of these cysts to interpret were the cystic clefts. They have merely retained the condition of childhood in the later periods of life and the cavity has become distended with colloid. The studies of Guizzetti ('26) including 72 pituitaries, from birth to 84 years, on the closure of the cleft help to interpret these cystic clefts. In 29 cases, from birth to 16 years, all of the clefts were open except two cases (a newborn and another 15 years). Of 9 cases, between 16 and 20 years, three specimens had the cleft still open. Of the 18 cases, from the age of 40 to 84, 16 had the fissure closed, one had the fissure partly closed and one from a 51-year old male had the cleft wide open and distended with colloid. Guizzetti found abundant colloid in the open cleft in all cases over 20 years of age and he believed that the colloid contributed to keeping it open.

One of our pituitaries (M218) with a cystic cleft was from a 17-year old girl whose death was due to embolism, the other two, M100, a 65-year old male, and M217, a 40-year old male, were both suicides. We agree with Guizzetti that the

distended condition of these clefts is due, at least in part, to the colloid and other secretory material accumulated there.

We believe that the ciliated cysts were derived, directly or indirectly, from Rathke's cleft. This interpretation was supported by the fact that many of them communicated with the cleft, or follicles derived from the cleft. The absence of such a communication does not invalidate such a conclusion for it is very probable that ciliated and mucous cells derived from the cleft may arise at a later date from partially or completely differentiated anterior lobe cells. This conclusion is supported by the studies of Severinghaus ('38, p. 102) who found what he called large colloid cysts in the anterior lobe of the dog. Although these cysts did not communicate with the cleft he frequently found them lined by ciliated columnar epithelium and mucus-secreting cells. At times these cysts were lined by normal chromophobes, acidophils and basophils. He also says (p. 103) "Some of the chromophobes had definite ciliated borders." This study showed that chromophobes may undergo metaplasia, and form cilia, however there is doubt as to whether or not such modified cells should still be referred to as chromophobes.

The paper by Bayoumi ('48) suggested another clue to the precursors of these cyst lining cells. In a preliminary note, based on an examination of 200 human pituitaries of all ages, he found columns of high columnar cells along the posterior border of pars anterior and near the cleft which he interpreted as fetal cells. Judging by his histological description and their appearance (figs. 1 and 2) one is led to postulate that cells similar to these were the precursors of the ciliated and mucus-secreting cells lining the cysts in our specimens.

The single multilocular cyst, in our series (M248) from a 3.5-year old female, is the result of disintegration. This conclusion is based on the necrotic anterior lobe cells adjacent to the cyst area and in the smaller peripheral cysts.

The small persistent canal was found in a 40-year old male (M115) whose death was attributed to progressive

paralysis. The location of this cyst along the upper aspect of the pituitary near the stalk, associated with the capsule and the nature of the epithelium led us to conclude that this cyst belonged to the hypophyseal stalk tumors.

None of the cysts described in this paper were similar to the large clefts described by Shanklin ('46b) in the human neurohypophysis.

Classification. Investigators who studied these tumors in lower animals referred to them merely as pituitary cysts and made no attempt to classify them. Writers dealing with human material, obtained by surgical intervention or at autopsy, have attempted to classify them. In general they fall into two major categories: those derived from Rathke's cleft, directly or indirectly, and those associated with the hypophyseal stalk. Both varieties may be of clinical importance.

Some good examples of those derived from Rathke's cleft are case IV of Duffy ('20) and the case of Frazier and Alpers ('34). Good examples of hypophyseal stalk cysts are cases I, II and III of Duffy ('20).

We propose the following classification for the cysts derived, directly or indirectly, from Rathke's cleft.

Pituitary cysts

Rathke's cleft cysts

- | | |
|-----------------|---------------------|
| 1. Cystic cleft | 2. Follicular cysts |
| Partial | Simple |
| Complete | Compound |
| | Multiple |

The above classification does not include hypophyseal stalk cysts, nor multilocular cysts which result from necrosis of the follicles.

In conclusion we can state that a study of the incidence of pituitary cysts showed that they occur in amphibians, reptiles, birds and mammals including man. Their frequency, however, was extremely variable in the different animals. The most frequent sites were the pars anterior and the pars intermedia, with an occasional diverticulum, in man penetrating into the infundibular process. In almost all the animals

where these cysts have been described the lining cells showed considerable variation in their morphology. Frequently parts of the cyst wall were devoid of lining cells; this was particularly true of the human. Cells varying from simple squamous to cuboidal and simple or stratified columnar may be present.

Usually patches of well developed cilia were found on some of these cells in all the animals. Mucous cells, often with the typical goblet shape, were a common finding among the ciliated cells. We believe that the lining cells of all these cysts from amphibia to man were essentially similar and were derived, directly or indirectly, from Rathke's cleft. A proposed classification is given for cysts derived from Rathke's cleft.

SUMMARY

1. One hundred human pituitaries varying from the newborn to 86 years of age were examined for cysts.
2. Cysts were found in 13 specimens, 11 of them above the age of 20 years.
3. Most of these cysts were considered as derivatives of Rathke's cleft and fell into two major categories: cystic clefts and follicular cysts.
4. Areas of ciliated cells, interspersed with mucous secreting cells, were frequently found in the follicular cysts.
5. The cystic clefts were a retention of the fetal and childhood condition into the adult state.
6. The essential similarity was stressed of follicular cysts in the human pituitary and cysts having the same histological characteristics found in amphibians, reptiles, birds and mammals.
7. A suggested classification is given of cysts derived from Rathke's cleft.

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PLATE 1

EXPLANATION OF FIGURES

All photomicrographs are of sections of the human pituitary, and except where especially noted, are stained with hematoxylin and eosin.

1 Section through pituitary M92 including a follicular cyst in the distal part of the pars anterior. $\times 6.3$

2 Section through pituitary M98 including a follicular cyst near the center of the pars anterior. $\times 6.3$

3 Section through pituitary M204 including a follicular cyst on the proximal side of the pars anterior. $\times 6.3$.

4 Section through pituitary M218 including a complete cystic cleft. $\times 6.3$.

5 Section through pituitary M230 including a follicular cyst in the proximal part of the pars anterior. $\times 6.3$

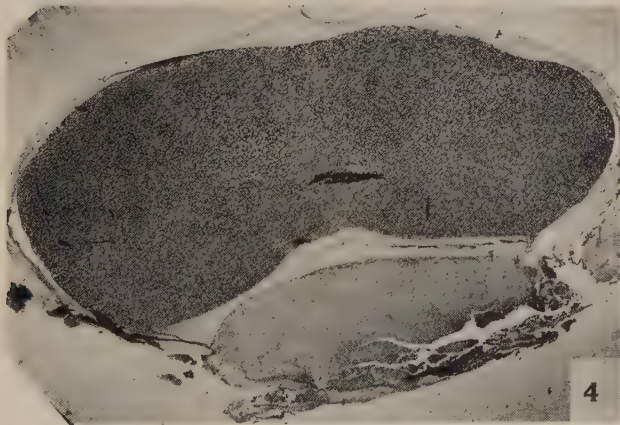
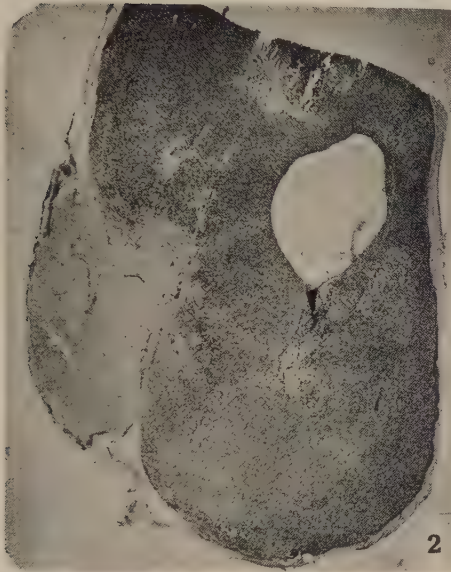
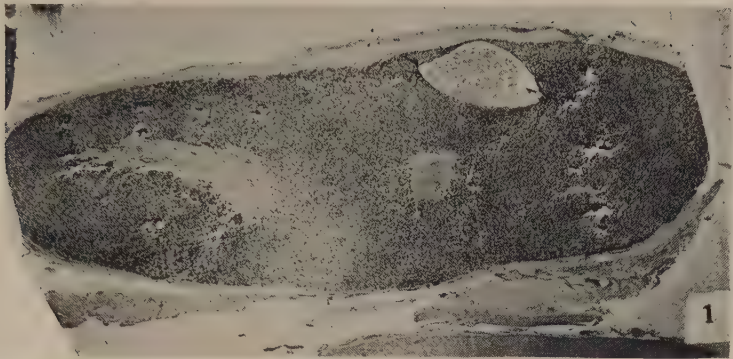


PLATE 2

EXPLANATION OF FIGURES

6 Section through a multilocular cyst in pituitary M248 situated in middle of pars anterior. $\times 180$.

7 Section through a follicular cyst in pituitary M101 located in pars anterior. $\times 350$.

8 Part of same section as in figure 7 more highly magnified. Note in both goblet cells, ciliated epithelium and the more darkly stained material overlying the lining cells. $\times 910$.

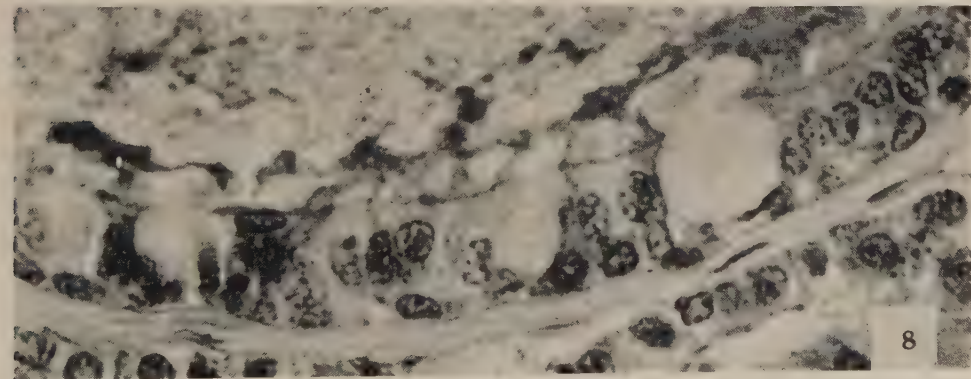
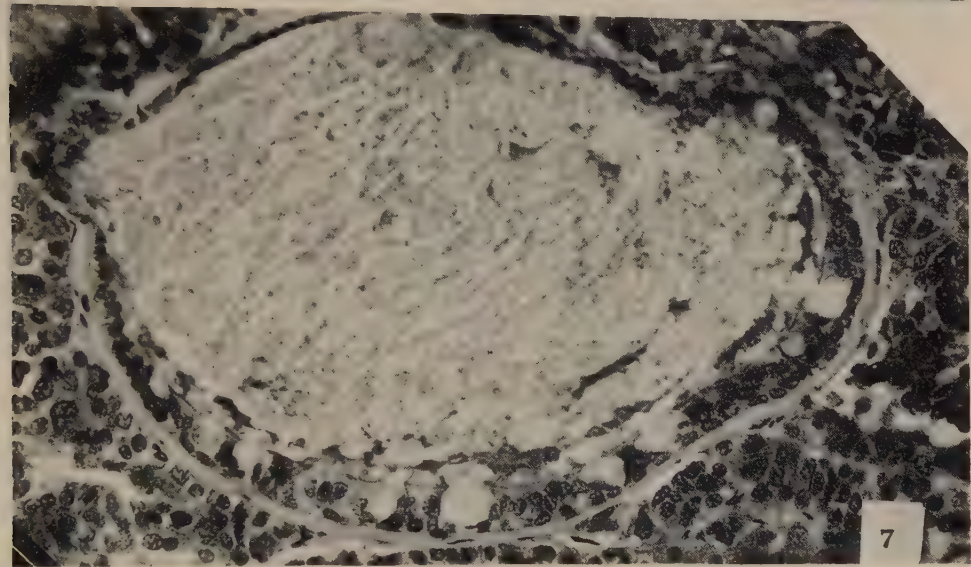
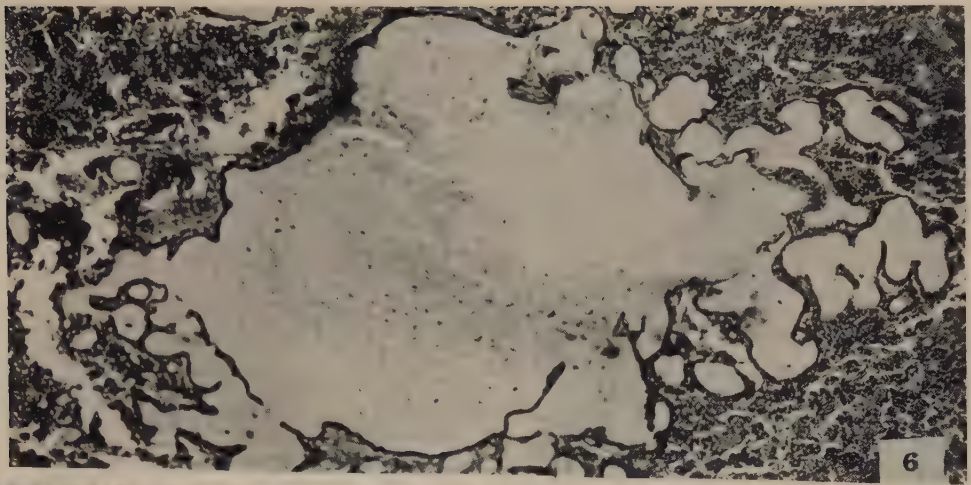


PLATE 3

EXPLANATION OF FIGURES

- 9 Section through cyst contents of pituitary M98.
- 10 Section through pituitary M204 including an area of ciliated epithelium and mucus-secreting cells. The more lightly stained areas in the lumen of the cyst are mucus material in the colloid. $\times 300$.
- 11 Section through pituitary M248 showing glands of Erdheim to the right with a duct opening into the cyst to the left. $\times 180$.
- 12 Section through pituitary M115 including the smaller part of the persistent canal. $\times 275$.
- 13 Section of pituitary M115 including expanded part of the persistent canal. $\times 275$.

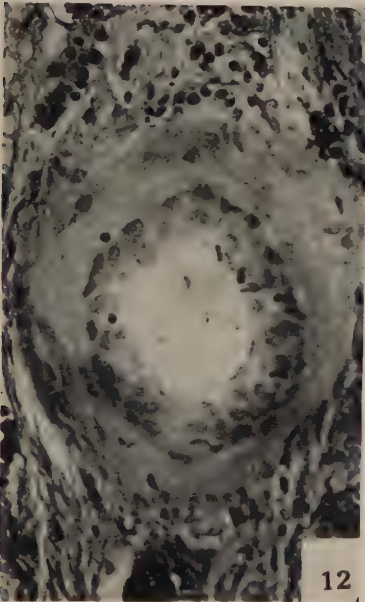
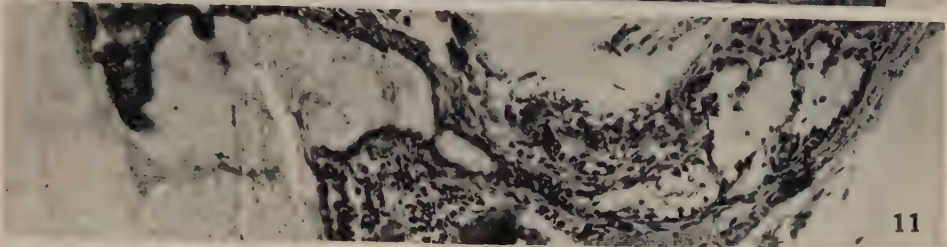
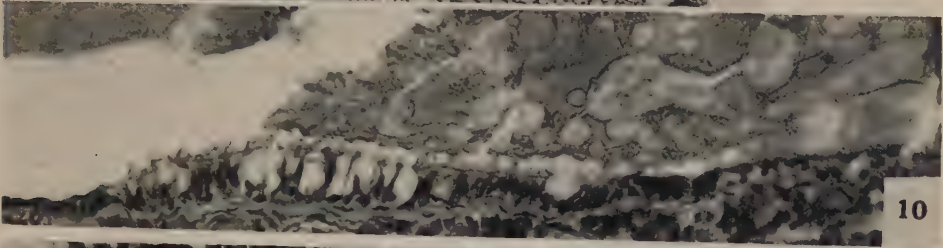
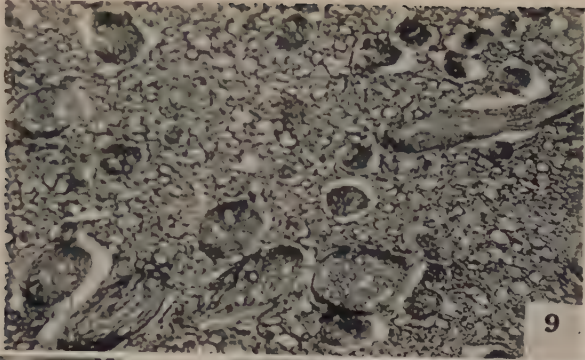


PLATE 4

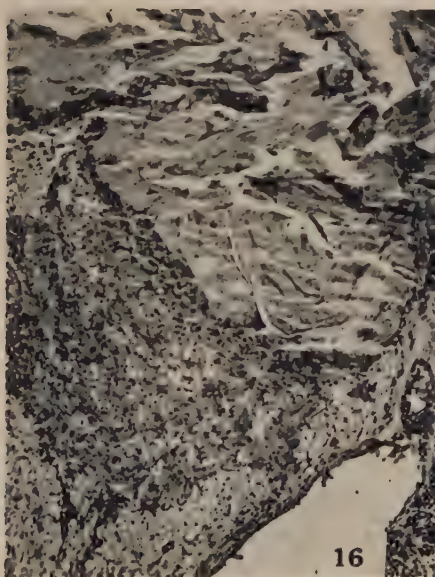
EXPLANATION OF FIGURES

14 Section of pituitary M183 including a follicular cyst in proximal part of pars anterior. $\times 35$.

15 Section through pituitary M92 including a follicular cyst in distal part of pars anterior. Mallory stain. $\times 35$.

16 Section through pituitary M204 showing a cyst that has invaded the pars nervosa. The lower part of this cyst wall is bounded by an area of tumorette cells. $\times 75$.

17 Section through pituitary M204 including a cyst. Note small area of tall epithelial cells lining part lower border. The dark areas in the cyst lumen are mucus material in the more lightly stained colloid. Mallory stain. $\times 75$.



TISSUE CULTURE STUDIES OF CILIATED NASAL MUCOSA IN MAN¹

PRELIMINARY REPORT

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Tissue Culture Laboratory, University of Texas Medical Branch

TWELVE FIGURES

Direct microscopic examination of living ciliated cells obtained from human nasal mucosa would seem to offer a useful tool for investigative purposes. Observations of their internal cellular structure together with a study of normal dynamics and response to various stimuli are highly desirable. Proetz and Pfingsten ('36 and '39) have reported successful cultivation of the ciliated nasal mucosa of the guinea pig fetus. The desquamated cells of the buccal mucosa of man have been described by Albertini ('46) and Ralph ('47) with the use of the phase-contrast microscope. With similar technique Lewis, Pomerat and Ezell ('49) have studied cells migrating from explants of human epidermis. Much interest has been shown in current articles in the study of direct smears of desquamated cells from the respiratory tree particularly with references to tumor diagnosis (Woolner and McDonald, '49). The purpose of the present work was the elaboration of a simple tissue culture technique, by means of which minute biopsy specimens of human ciliated mucosa could be observed with phase-contrast photomicrography and, particularly, with cinematography.

¹ Aided by a grant from the American Cancer Society CP 12 A.

MATERIALS AND METHODS

Tissue was obtained from the nasal mucosa by means of a small caliber biopsy punch. The average size of specimens was approximately 8 mm square and yielded 8-12 explants each about 2 mm square. Satisfactory biopsies were obtained with a minimum of pain and bleeding without the use of topical anaesthetics or vaso-constricting agents. Outpatients in the Otolaryngology Department as well as laboratory personnel were used as subjects. Normal nasal mucosa was employed for this study although observations on allergic tissue using the same technique are now in progress.

Initial failure to obtain outgrowth was overcome when topical anaesthetics and vaso-constrictors were not used in preparing the nasal mucosa for biopsy. Only occasional specimens removed with these precautions bore any cilia. Biopsy specimens from the mucosa overlying the posterior part of the middle turbinate revealed actively beating cilia in the majority of cultures both in the explant and the outgrowing sheet.

Specimens were placed in Tyrode's solution immediately after removal from the nose in order to prevent drying. Although taken from an area that was primarily contaminated, each specimen was handled with aseptic precautions from the time that it was removed from the nose on through the entire procedure. Approximately 450 explants from 12 different subjects were cultured and examined. Biopsy specimens were cut with very sharp knives in order to minimize trauma.

Each individual explant was embedded in a clot formed by the addition of one part of cockrel plasma containing 2000 units of penicillin per milliliter to a mixture of equal parts of embryonic extract from 7-day chicks and human ascitic fluid. A supernatant nutrient fluid was made up of two parts of ascitic fluid, one part of a mixture of embryonic extract and Tyrode's solution, was used for Roller tube and Carrel flask cultures. The fluid phase was changed once each week. Preparations were set up in hanging drop slides, roller tubes,

Carrel flasks and Gey's metal phase slides. Liquefaction of the clot, presumably due to proteolytic enzymes caused considerable difficulty. Early remounting of the explant or patching was necessary in order to prevent their destruction.

OBSERVATIONS

A thin sheet of epithelial outgrowth ordinarily migrated from the explanted tissue within two to three days and reach a maximum in 5 to 7 days (fig. 1). Between the 8th and 10th day outgrowth generally stopped and was followed by rounding up of the cells in the next three to 5 days. Migration in roller tubes was usually more extensive than in hanging-drop preparations. Ciliary activity persisted at room temperatures for 4 weeks in a roller tube culture and for 8 days in a phase-contrast slide prepared without sterile precautions. No attempt was made at this time to determine longevity of cultures nor was any special effort made to maintain continuous growth by more frequent changes of supernatant fluid and other measures. A fibroblastic type of outgrowth appeared within 10–14 days in some cultures that had failed to produce a sheet of epithelial cells.

Cytological features of epithelial cells in migrating sheets are shown in figures 2–8. Cell boundaries were not easily seen but often consisted of dense bands of cytoplasm (figs. 2 and 7). At the free edge the cytoplasm formed a thin wavy undulating membrane sometimes showing vacuole formation and occasionally showing a pattern of irregular spikes (fig. 6). Nuclei (figs. 5–8) showed sharply defined nuclear membranes and 1 to 3 nucleoli of various shapes and sizes in a clear field of nucleoplasm. Mitochondria were generally of the long filamentous type (figs. 3 and 4) but with age they tended to show evidences of degeneration (fig. 5). Fatty droplets (fig. 7) increased in size especially if the nutrients were not replenished (fig. 8).

Ciliary action was observed in the various types of preparations although in roller tubes successful cultures of this kind were more consistent than with any of the other methods.

Ciliary activity was absent in the explant of many cultures during the first hour or two after imbedding in the plasma clot but was soon reinitiated. As previously mentioned, the area of the nasal mucosa from which the biopsy specimen was obtained seemed to be the most important factor in obtaining actively beating cilia. No studies as yet have been made to determine directional characteristics of the ciliary action or to measure the speed and strength of ciliary motion. With phase-contrast microscopy cilia were seen to occur in tufts of variable size (figs. 9-12). The distribution of these tufts followed no constant pattern. A single patch was observed to appear on the surface of some cells (figs. 9 and 11). Several tufts were grouped to form larger patches of cilia involving 2-5 cells in some of the cultures (fig. 10). Specialized channels, formed by strips of ciliated cells extending though the midst of a large sheet of non-ciliated elements were observed in roller tube cultures.

DISCUSSION

In a chance observation on culture set-up for a phase-contrast microscopy in which no sterile precautions had been employed, actively beating cilia were seen at room temperature for a period of 8 days. One must consider the likelihood that the nasal mucosa possesses a high degree of natural antibacterial immunity. With special attention to pH, electrolyte balance, osmotic equilibrium, and other factors, a method of maintaining cultures of nasal epithelium for an indefinite length of time may be developed. However, for the purpose of observations proposed for the study of allergic reactions a culture maintained in a healthy state for a week should prove adequate. Cilia in living cultures tend to be vigorous and to withstand deleterious conditions. Thus the effects on ciliary action of various local agents commonly used in the nose might be examined to good advantage by the method described.

The study of the living cell under the phase-contrast technique together with moving picture records offers important additional end-points for experimental studies. Detailed observations on the mitochondria, nucleus, nucleoli as well as on the rate of epithelial outgrowth may prove to be instrumental in the detection of early signs of cell injury by agents such as toxins, bacteria, chemical agents and antigens. The method also suggests possibilities for the culture and study of other cells from the respiratory tract.

Culture of ciliated nasal epithelium was undertaken as one step in an experimental study of bacterial hypersensitivity in man. Previous work has dealt chiefly with tissue cultures of tissues from guinea pigs and rabbits that had been previously sensitized to tuberculosis by inoculation or by naturally occurring bacterial infection. Using *in vitro* technique Aronson ('31) studied the reaction of cells sensitized to tubercle bacilli, streptococci and horse serum. He demonstrated that the cells sensitized with horse serum (Aronson, '33) failed to show evidence of reaction on addition of the specific antigen. The cells taken from animals sensitized to the tuberculosis infection showed the most marked and persistent reaction. Cells from guinea pig infected with streptococcus showed an intermediate type of reaction. Studies are being conducted on atopic allergic tissues in which the specific sensitivity has been determined. Antigen titration studies along these lines are in progress.

SUMMARY

1. A technique for short term cultivation of human ciliated nasal epithelium is described.
2. Ciliated cells were observed to continue to show activity for 4 weeks with this method.
3. Studies of ciliary action and cellular structural detail were made with phase-contrast technique and cinematography.
4. The advantages of the method for the study of toxins, bacteria, chemical agents and antigens particularly in human allergy are briefly discussed.

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PLATE 1

EXPLANATION OF FIGURES

Figures 1 to 12 represent living cells from the human nasal mucosa cultivated *in vitro*. Figure 1 was taken with the light microscope while figures 2 to 12 are dark phase-contrast photomicrographs taken with a 1.9 mm Spencer objective and a 10 × Zeiss ocular. The magnification of all phase illustrations is approximately 1500 ×. All photographs were made of cultures incubated at 37°C. and with the exception of figure 8 were taken on the 5th day.

1 The dense central bar represents the explanted fragment of mucosa from which a single layer of closely packed cells had formed a migrating sheet. Roller tube culture magnified approximately 110 ×.

2 Characteristic grouping of cells in outwandering sheet. Note irregularities in the density of the cytoplasm between cells.

3 and 4 Shows mitochondrial pattern commonly seen near the nucleus and near the edge of the cell.

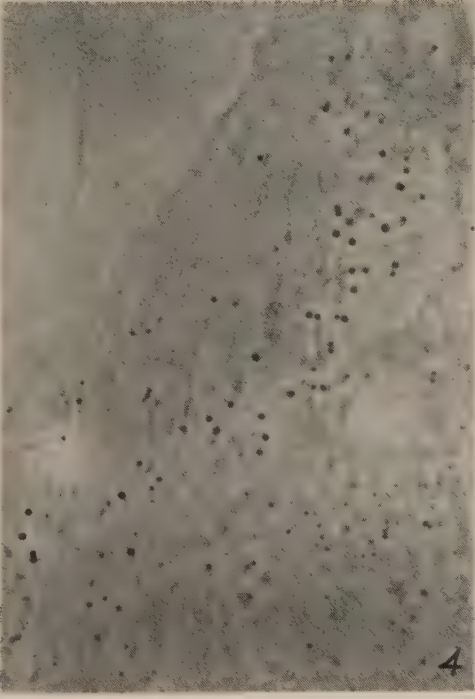
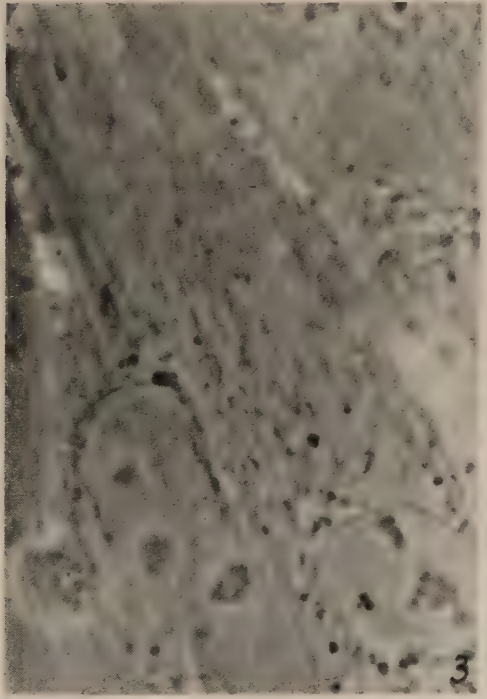
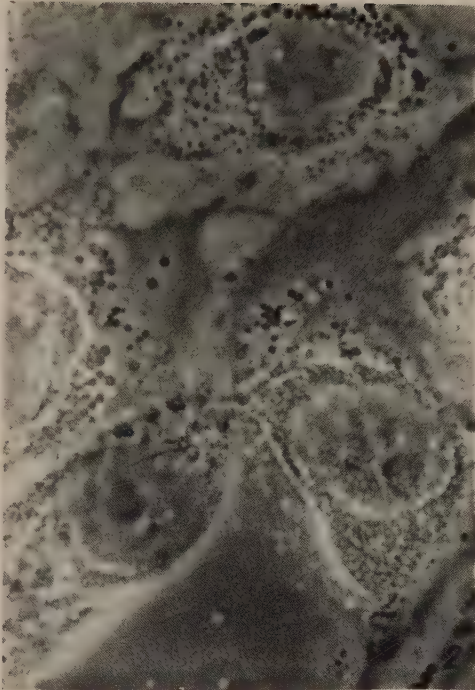
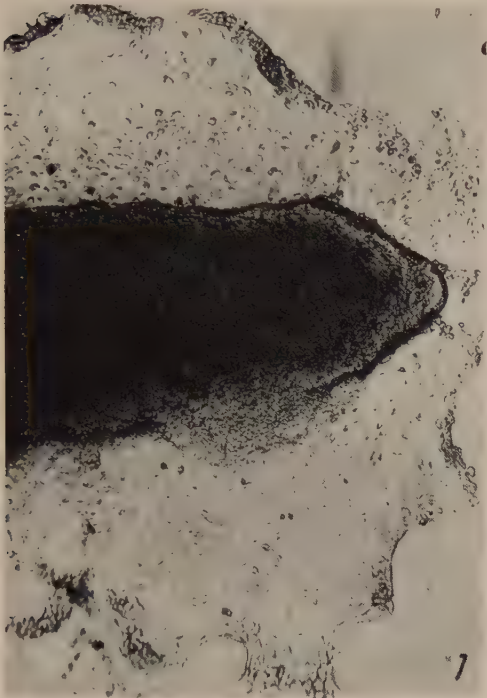


PLATE 2

EXPLANATION OF FIGURES

5 to 8 Nuclei of living cells from the nasal mucosa showing the form and arrangement of the nucleoli, the character of the sharply defined nuclear membrane and the distribution of various granules.

5 Mitochondria not in typical long filaments probably indicating injury.

6 Pattern of the membrane at the free edge of cells.

7 Fat droplets surrounding the nucleus of a cell showing clearly marked bands of cytoplasm at the points of contact with an adjacent element.

8 Conspicuous fatty droplets which form in cultures after 7 days when nutrient medium is not replaced.

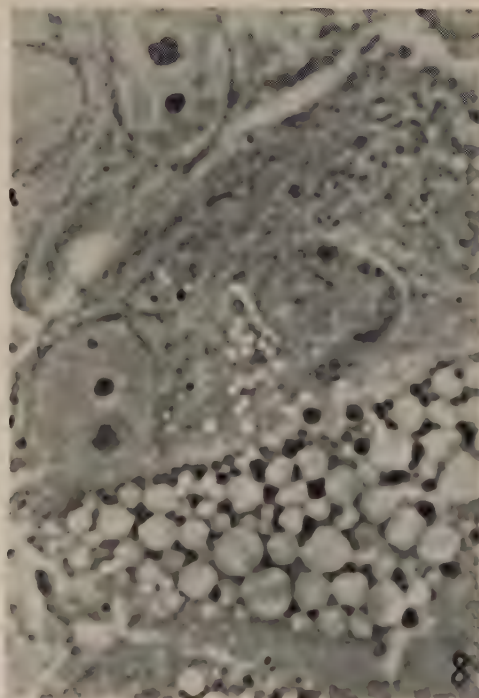
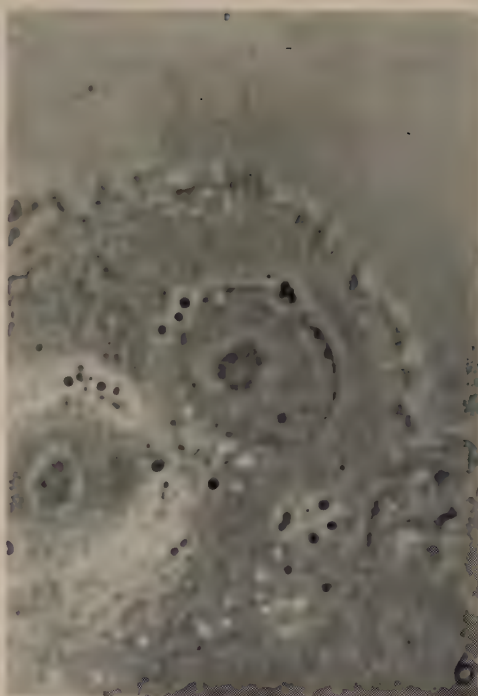
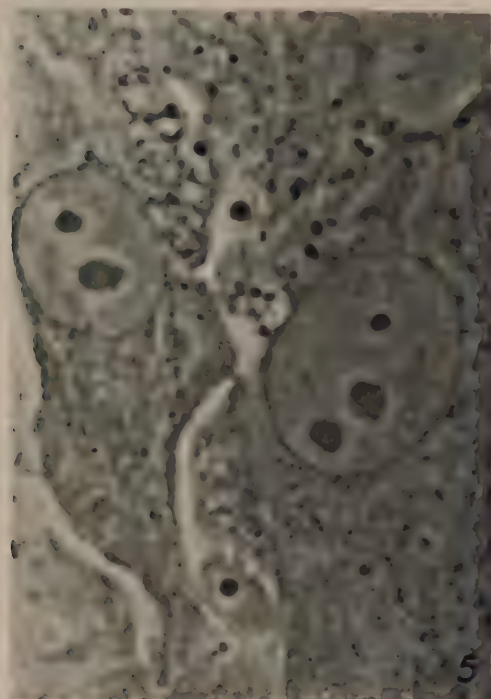
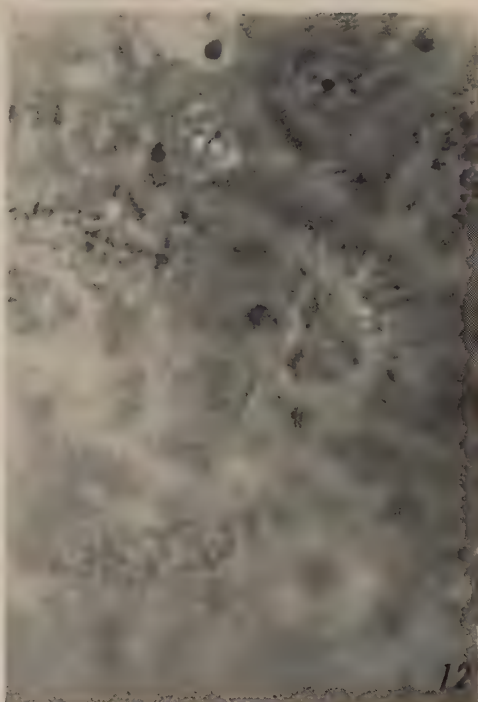
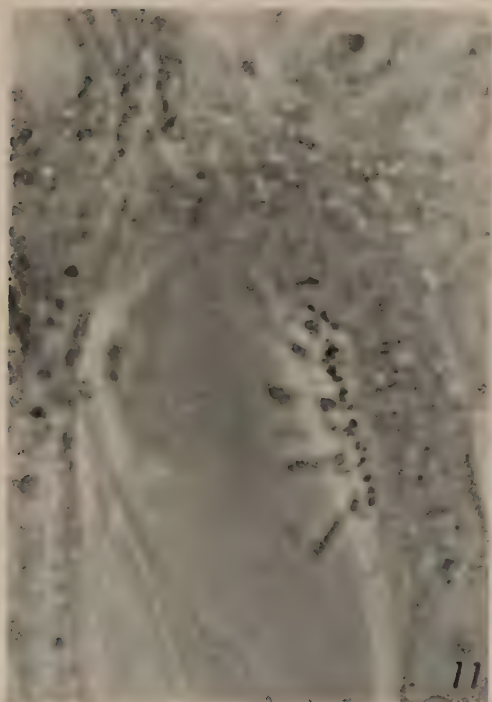
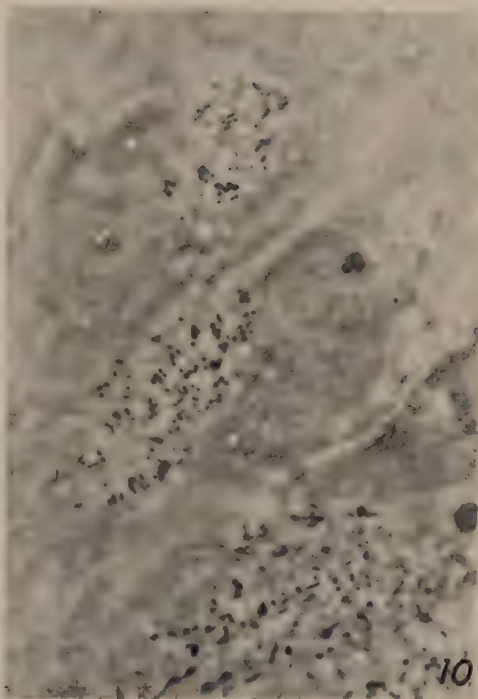
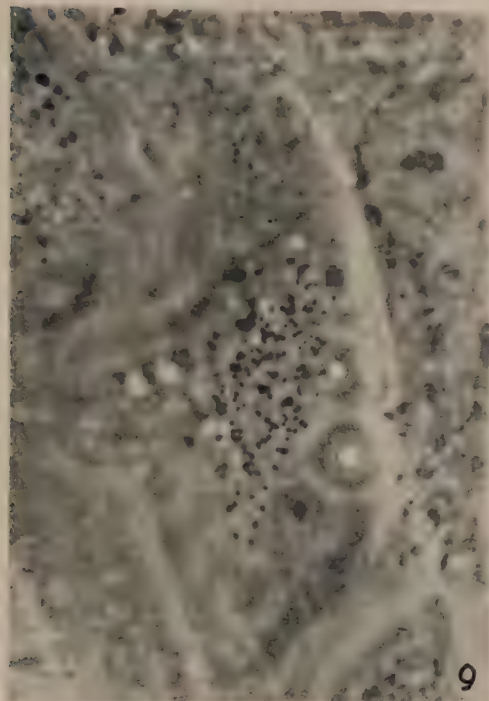


PLATE 3

EXPLANATION OF FIGURES

Figures 9-12 Appearance of cilia in living cells. Photographs were taken at a focal plane close to the base of the cilia so as to avoid blurring by movement (figs. 9 and 10) or by short exposures (figs. 11 and 12). All show arrangement of cilia in discrete patches as well as the relation of groups of cilia in adjacent elements.



THE EFFECTS OF ANTITHYROID DRUGS ON CHICK EMBRYOS ¹

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FOURTEEN FIGURES

INTRODUCTION

In 1946 Grossowicz found that chick embryos whose yolk sacs were injected with thiourea between the 7th and 17th days of incubation were delayed in hatching and their yolk sacs were not retracted. Some correlation existed between the dosage administered and the degree of retardation. Simultaneous treatment with thyroxine neutralized the action of the thiourea. These results were interpreted as suggesting "that in the chick embryo, as in other animals, the inhibiting effect of thiourea may be due to interference with normal thyroxine metabolism" (p. 152). Grossowicz, however, did not report any effects on body growth or describe the condition of the thyroid glands of his treated animals. The present experiments were therefore undertaken to determine whether growth would be modified and whether the thyroid glands would be affected by the administration of antithyroid drugs.

MATERIAL AND METHODS

Single doses of 0.5 cm³ of a 0.4% solution of thiourea ² and of a 0.1 or 0.2% solution of thiouracil ³ were injected by a

¹ An abstract of certain of these results was presented by title at the 1948 meeting of the American Association of Anatomists and appeared in *The Anatomical Record*, vol. 100, p. 729.

² Thiocarbamide (CS(NH₂)₂) purchased from Eimer and Amend.

³ Deracil (2-thio-6-oxypyrimidine), supplied by courtesy of the Lederle Laboratories, Inc., New York City.

modification of the methods of Cox ('41) and Grossowicz ('46) into the yolk sacs of White Plymouth Rock chick embryos on the following days: (1) 8th, (2) 14th, (3) 8th and 14th, (4) 8th, 14th and 18th, and (5) 18th days of incubation. The 8th day was chosen because it is prior to the appearance of colloid on the 10th (or 11th) day (Hopkins, '35; Woodside, '37; Martindale, '41) and the 14th because follicles are well formed by the 12th (or 13th) (Bradway, '29). From the 15th day on the gland has essentially the structure which it maintains until hatching except for follicular growth and enlargement (Hopkins, '35; Martindale, '41). Those injected on the 8th or 14th days were dissected every day from the 10th (or 15th) until the 21st and a few on the 22nd and 24th; those on the 18th day, on the 20th. The sets injected on both the 8th and 14th days were dissected on the 18th, 21st and 22nd days. Those with three injections were killed on the 21st day. Controls received 0.5 cm³ of distilled water or were not injected. Since there was no significant difference in these two control groups, their data were combined.

At dissection the embryos were freed from their extra-embryonic membranes and weighed to the nearest 0.1 gm (table 1). In some cases the left wing and the left leg were cut off, preserved in 10% formalin and later macerated in 5% KOH, stained in alizarin red S and cleared. Measurements of the tibiotarsus of some legs were made with a vernier caliper to the nearest 0.1 mm (table 2). The thyroids of the chicks were dissected out and weighed on a precision balance (table 3), fixed in Bouin's fluid—(a few were put in Helly's fluid),—sectioned at 5 μ and stained in either Delafield's or Mayer's hematoxylin and Mallory's connective tissue stain or eosin. The height of the follicular epithelium of the majority of the thyroid glands from chicks 14 days old or older was measured with a Leitz ocular micrometer at a magnification of 970 times. Five sections separated by at least 5 others were chosen from the middle of the gland and two cells opposite each other in 10 follicles across the diameter of each section were measured. Mean cell height and its standard de-

viation were computed for each gland and the average mean cell height determined for each group (table 4).

OBSERVATIONS

Chicks receiving antithyroid drugs were delayed in hatching if the eggs containing them were incubated to hatching date or beyond. No retraction of yolk sac or hatching occurred in the 15 thiourea-injected eggs (21 to 24 days of incubation). The 5 injected with thiouracil, however, varied. Two, examined on the 21st and 22nd days, had their yolk sacs wholly or partially retracted, two others on the 22nd day showed no yolk sac resorption and one hatched on the 24th day.

From an examination of table 1, it is evident that in general the average body weight of chicks injected with thiourea or thiouracil was less than that of controls injected with distilled water or left untreated. Occasionally there were exceptions such as the group given one injection of thiourea on the 18th day and autopsied on the 20th, the set injected on the 14th day and dissected on the 15th, and two cases in which thiouracil was administered. The fact that the number of chicks dissected each day was small may account in part for the variability in the percentage decreases, but the greatest decreases usually occurred in those chicks dissected on the 18th day and thereafter (table 1, figs. 1 and 2). The decreases in body growth did not seem to be correlated with the number of injections (one, two or three) or with total dosage (2.0, 4.0, or 6.0 mg of thiourea; 0.5-1.0, 1.0-2.0, or 1.5-3.0 mg of thiouracil). The single dose of thiourea given on the 8th or the 14th day produced very similar effects although the percentage decreases on the 15th, 17th, 19th, 21st and 22nd days were considerably greater in the chicks exposed to thiourea from the 8th day (table 1). The single dose of thiourea on the 18th day caused no decrease; in fact the treated chicks were larger than the controls. In general, thiouracil did not influence body growth as much as thiourea.

Another evidence of retarded growth was the lessened ossification in the limbs (figs. 3 and 4). Measurements of the

tibiotarsus of some embryos showed that after the 15th day the figures for experimental chicks were less (with two exceptions) than those for controls (table 2).

The changes in the thyroid glands of the chick embryos following yolk sac injections of antithyroid drugs were evi-

TABLE 1

Average body weights of chick embryos injected with thiouracil or thiourea at various ages of incubation compared with controls injected with distilled water or untreated

AGE AT KILLING	CONTROLS		THIOURACIL-INJECTED			THIOUREA-INJECTED		
	Nos. of chicks	Av. body wt.	Nos. of chicks	Av. body wt.	% difference	Nos. of chicks	Av. body wt.	% difference
days		gm		gm			gm	
<i>Injected at 8 days of incubation</i>								
10	2	3.5	1	3.5	0.0	3	3.4	— 2.9
11	7	4.0	2	3.5	— 12.5	6	3.9	— 2.5
12	4	3.8	1	3.1	— 18.4	3	3.7	— 2.6
13	3	5.9	..	..	..	3	5.3	— 10.2
14	4	9.6	1	10.4	+ 8.3	3	9.1	— 5.2
15	4	10.3	1	9.3	— 9.7	3	8.7	— 15.5
16	4	13.7	1	10.0	— 27.0	3	12.5	— 8.8
17	3	18.7	1	15.5	— 17.1	2	14.7	— 21.4
18	3	20.6	2	18.5	— 10.2	3	16.8	— 18.4
19	4	20.4	..	..	..	3	14.4	— 29.4
20	3	23.4	1	17.5	— 25.2	2	18.5	— 20.9
21	12	34.4 ¹	..	..	..	2	20.5	— 40.4
22	2	36.8 ¹	1	29.7	— 19.3	1	16.5	— 55.2
24	..	..	1	29.8 ¹	— 19.0	2	22.3	— 39.4
<i>Injected at 14 days of incubation</i>								
15	4	10.3	..	..	..	3	10.6	+ 2.9
16	4	14.0	1	13.7	— 2.1	2	11.4	— 18.6
17	3	17.1	1	15.3	— 10.5	3	14.4	— 15.8
18	2	24.5	..	..	..	2	17.6	— 28.2
19	4	26.5	2	22.7	— 14.3	4	21.0	— 20.8
20	3	27.4	1	18.0	— 34.3	3	18.4	— 32.8
21	12	34.4 ¹	1	37.1 ¹	+ 7.8	2	21.6	— 37.2
22	2	36.8 ¹	1	30.1	— 18.2	1	29.0	— 21.2
<i>Injected at 8 and 14 days of incubation</i>								
18	1	20.0	1	19.0	— 5.0	3	18.7	— 6.5
21	12	34.4 ¹	..	..	..	1	23.0	— 33.1
22	1	36.1 ¹	1	23.0	— 36.3	2	22.1	— 38.8
<i>Injected at 8, 14, and 18 days of incubation</i>								
21	3	29.2	..	..	..	4	20.0	— 31.5
<i>Injected at 18 days of incubation</i>								
20	4	35.0	..	..	..	2	39.4	+ 12.6

¹ Figure obtained by subtracting 7 gm for the weight of the yolk sac from the weight of the chick which either had hatched or had its yolk sac retracted.

dent in the general appearance of the glands at the time of dissection, in their weights and in the histological picture they presented compared with those of controls. Especially during the last days of incubation, the experimental glands were more hyperemic, larger and rounder than those of the controls. The average absolute thyroid weights were consistently increased except in chicks injected with thiourea

TABLE 2

Length of the ossification centers of the tibiotarsus in chick embryos injected with thiouracil or thiourea at various ages of incubation and killed from the 15th to the 22nd day of incubation compared with controls injected with distilled water or untreated

AGE AT KILL- ING	AGE AT INJEQ- TION	CONTROLS		THIOURACIL-INJECTED			THIOUREA-INJECTED		
		Nos. of chicks	Length of tibio- tarsus	Nos. of chicks	Length of tibio- tarsus	% differ- ence	Nos. of chicks	Length of tibio- tarsus	% differ- ence
<i>days</i>	<i>days</i>		<i>mm</i>		<i>mm</i>			<i>mm</i>	
15	8	2	4.9	1	5.6	+ 14.3	1	6.6	+ 34.7
16	8	2	8.5	1	5.2	— 38.8	1	8.4	— 1.2
17	8	2	12.9	1	12.2	— 5.4	1	11.4	— 11.6
18	8,14	1	15.5	..	..	..	2	15.0	— 3.2
19	8	3	17.8	..	..	..	2	12.0	— 32.6
	14	1	18.6	1	19.5	+ 4.6	1	18.6	0.0
20	8	2	18.1	1	16.1	— 11.1	1	16.8	— 7.2
21	8	1	21.3	..	..	..	1	17.6	— 17.4
	8,14,18	2	21.6	..	..	..	1	17.5	— 19.0
22	8	2	20.8	1	19.0	— 8.7	1	17.7	— 14.9

on the 8th and autopsied on the 11th day and in those injected on the 18th and autopsied on the 20th day (table 3). These exceptions may be explained by the very early developmental stage of the former and the brevity of exposure of the latter allowing colloid release but no general enlargement. Occasionally the increases were small as on the 13th day after an injection of thiourea on the 8th day, or on the 15th day after a single injection on either the 8th or the 14th day or on the 17th and 20th days after an injection of thiouracil on the 8th day. It is possible that data on a larger number of chicks would iron out some of the variability in response.

From the 18th day on, a single injection of thiourea on the 14th day evoked larger percentage increases than one given on the 8th and the same was true in the thiouracil group from the 16th day on, where figures are available. Two injections of thiourea (on the 8th and 14th days) produced by the 18th day a percentage increase higher than did one injection on

TABLE 3

Average absolute weights of thyroids of chick embryos injected with thiouracil or thiourea at various ages of incubation compared with controls injected with distilled water or untreated

AGE AT KILLING	CONTROLS		THIOURACIL-INJECTED			THIOUREA-INJECTED		
	Nos. of chicks	Av. thyroid wt.	Nos. of chicks	Av. thyroid wt.	% difference	Nos. of chicks	Av. thyroid wt.	% difference
days		mg		mg			mg	
<i>Injected at 8 days of incubation</i>								
10	2	0.12	1	0.25	+ 108.3	2	0.30	+ 150.0
11	6	0.27	2	0.36	+ 33.3	6	0.25	— 7.4
12	4	0.65	1	0.82	+ 30.2	3	0.89	+ 41.3
13	3	1.11	..	...	...	3	1.18	+ 6.3
14	4	1.50	2	2.64	+ 76.0	3	2.77	+ 84.7
15	4	2.50	1	4.21	+ 68.4	3	2.63	+ 5.2
16	4	3.42	1	4.72	+ 38.0	3	6.26	+ 83.0
17	3	4.12	1	4.37	+ 6.1	2	7.83	+ 90.0
18	3	5.07	2	6.86	+ 35.3	3	8.72	+ 72.0
19	4	4.00	..	...	...	3	5.57	+ 39.3
20	3	3.58	1	3.82	+ 6.7	2	8.11	+ 126.5
21	11	9.57	..	...	...	2	16.36	+ 71.0
22	2	8.54	1	10.81	+ 26.6	1	13.84	+ 62.1
24	..	...	1	16.30	+ 90.9	2	25.11	+ 194.0
<i>Injected at 14 days of incubation</i>								
15	4	1.22	..	...	...	3	1.43	+ 17.2
16	4	1.45	1	2.38	+ 64.1	2	2.09	+ 44.1
17	3	2.38	1	3.92	+ 64.7	3	3.80	+ 59.7
18	2	1.78	..	...	...	2	7.97	+ 347.8
19	4	6.37	2	10.02	+ 57.3	4	12.14	+ 90.6
20	3	3.41	1	9.47	+ 177.7	3	9.56	+ 180.4
21	11	9.57	..	...	...	2	22.20	+ 132.0
22	2	8.54	1	18.32	+ 114.5	1	15.09	+ 76.7
<i>Injected at 8 and 14 days of incubation</i>								
18	1	4.54	1	8.71	+ 91.9	3	10.96	+ 141.4
21	11	9.57	..	...	...	1	12.19	+ 27.4
22	1	10.18	1	25.32	+ 148.7	2	24.00	+ 135.8
<i>Injected at 8, 14, and 18 days of incubation</i>								
21	3	4.30	..	...	...	3	8.58	+ 99.5
<i>Injected at 18 days of incubation</i>								
20	4	7.34	..	...	...	2	6.55	— 10.8

the 8th, but lower than one on the 14th. On the 21st day, the percentage increase was lower after two injections than after one, but on the 22nd day the reverse was true. Similarly, two injections of thiouracil were more effective by the 18th day than a single one given on the 8th and, by the 22nd day, more effective than a single one on either the 8th or the 14th. Three injections of thiourea (on the 8th, 14, and 18th days) caused a greater effect by the 21st day than one on the 8th or two on the 8th and 14th, but not more than one on the 14th. The differences in response of the thyroids to thiourea and thiouracil were not consistent. An injection of thiouracil on the 8th day usually caused smaller increases in thyroid weights at least from the 16th day on than thiourea did. A single treatment on the 14th day, however, gave greater increases in chicks autopsied on the 16th, 17th and 22nd days, but they were smaller in those autopsied on the 19th and 20th days than with comparable thiourea injections. Two injections produced similar inconsistencies where figures are available, as on the 18th and 22nd days.

Data on relative thyroid weights (thyroid weight as a percentage of body weight) in chicks treated with antithyroid drugs compared with controls emphasized both the increase of thyroid weight and the decrease of body weight. The relative thyroid weight in the treated chicks was consistently much greater (one exception) than that in the controls, especially toward the end of the incubation period when increased absolute thyroid weight was associated with a decrease in body weight.

Histological changes prior to the 14th day in the thyroids of chicks given antithyroid drugs were somewhat difficult to analyze. On the 10th through the 13th days, while there was typically an increase in the size of the thyroid gland and mitoses were usually numerous, the differentiation of the gland seemed retarded. The pattern was more cord-like than in controls and pin points of colloid were less abundant. By the 13th day, however, the colloid that was present was more vacuolated than that in controls. From the 14th day on, the

histological appearance of experimental glands differed considerably from that in controls. The follicular epithelium ranged from medium to high cuboidal or even columnar, colloid was more vacuolated and less abundant, and mitoses were more numerous, whereas in control glands, the epithelium was squamous to low (rarely medium) cuboidal, colloid was homogeneous and abundant (figs. 5-14).

The appearance of the epithelium was confirmed by measurements. The thyroid epithelial height in treated chicks always exceeded that in controls except in a thiouracil-injected chick autopsied on the 20th day. Percentage increases were marked and, in thiourea-injected chicks, usually rose in an ascending curve (table 4). Those for chicks treated on the 8th day and autopsied on the 18th and 19th days were lower than on preceding or subsequent days, but numbers were too small to be certain that these exceptions were significant. It was true, however, that toward the end of incubation there was a drop in epithelial height in control thyroids, too. Thiouracil typically produced a lesser and more variable response than thiourea.

The increases in thyroid cell heights after one injection of thiourea on the 8th or 14th day and after one, two and three injections presented an inconsistent picture as did the figures for the thyroid weights. With the exception of the figure on the 20th day, a single injection given on the 8th day produced cells as high or higher than one on the 14th. The percentage increases varied more, but were also greater on the 15th, 16th, 17th and 21st days. The effects of two injections (on the 8th and 14th days) at autopsy on the 18th day were greater than after one on either the 8th or the 14th day, but at autopsy on the 21st day the reverse was true. Three injections (on the 8th, 14th and 18th days) produced higher cells at autopsy on the 21st day than two (on the 8th and 14th days) or one on either the 8th or the 14th day. However the percentage increase (128.7%) was lower than the respective comparable figures probably because the cells of the controls were so high.

TABLE 4

Mean cell height in μ (100 cells in each gland measured) of thyroids of chicks injected with thiouracil or thiourea at various ages of incubation compared with that in controls injected with distilled water or untreated

AGE AT KILLING	CONTROLS		THIOURACIL-INJECTED			THIOUREA-INJECTED		
	Nos. of chicks	Mean thy. cell ht.	Nos. of chicks	Mean thy. cell ht.	% difference	Nos. of chicks	Mean thy. cell ht.	% difference
<i>days</i>		μ		μ			μ	
<i>Injected at 8 days of incubation</i>								
14	2	3.97	1	7.57	+ 90.7	3	6.68	+ 68.3
15	4	3.94	1	7.04	+ 78.7	3	8.25	+ 109.4
16	4	3.87	1	7.57	+ 95.6	3	8.55	+ 120.9
17	3	4.14	1	6.74	+ 62.8	2	9.37	+ 126.3
18	2	4.06	1	7.09	+ 74.6	2	7.80	+ 92.1
19	3	4.85	..	...	...	3	8.55	+ 76.3
20	3	4.01	1	2.97	- 25.9	2	8.89	+ 121.7
21	5	3.09	..	...	...	1	9.28	+ 200.3
22	1	2.82	..	...	...	..	...	...
24	..	...	1	4.45	...	2	7.70	...
<i>Injected at 14 days of incubation</i>								
15	4	3.99	..	...	...	3	6.51	+ 63.2
16	4	4.22	1	7.17	+ 69.9	2	8.05	+ 90.8
17	3	4.29	1	6.57	+ 53.1	3	8.52	+ 98.6
18	..	...	..	...	...	1	7.80	...
19	4	3.32	2	3.74	+ 12.7	4	7.95	+ 139.5
20	3	3.87	1	5.56	+ 43.7	3	10.19	+ 163.3
21	5	3.09	..	...	...	1	8.88	+ 187.4
<i>Injected at 8 and 14 days of incubation</i>								
18	1	4.18	1	6.69	+ 60.0	3	8.73	+ 108.9
21	5	3.09	..	...	...	1	7.50	+ 142.7
22	..	...	1	6.64	...	2	9.06	...
<i>Injected at 8, 14, and 18 days of incubation</i>								
21	3	4.32	..	...	...	3	9.88	+ 128.7
<i>Injected at 18 days of incubation</i>								
20	2	3.10	..	...	...	2	4.18	+ 34.8

DISCUSSION

The main effects on chick embryos of the administration of antithyroid drugs, thiourea and thiouracil, during incubation were (1) retardation of hatching and lack of yolk sac retraction, (2) decreased growth, and (3) enlargement of the

thyroid with its associated hyperplasia and hypertrophy. The first of these effects was similar to that reported by Grossowicz ('46) but less striking since the eggs were only allowed to go three days beyond normal hatching compared with his 10 days. He reported a correlation between the time of injection (the 7th to the 17th day) and dosage administered (0.3 to 3.0 mg of thiourea), and degree of retardation, but in the present study 2.0, 4.0 or 6.0 mg of thiourea proved equally effective and beginning treatment on the 8th or 14th day made no apparent difference. Grossowicz was able to neutralize the retardation of hatching by simultaneous administration of thyroxine so the thiourea had probably produced a hypothyroid state as antithyroid drugs do in other animals (Astwood, '45; Astwood et al., '43; review, Charipper and Gordon, '47). A similar delay in yolk sac retraction and in hatching occurred in chicks hypophysectomized during incubation (Fugo, '40). The fact that the thyroid was not normally developed and not normally functioning as judged by colloid formation makes the possibility of hypothyroidism following pituitary loss the essential factor in this delay.

The evidence for the second effect, decreased growth, was mainly the lowered body weight and shorter limbs (judged by measurements of the tibiotarsus) in the thiourea- and thiouracil-injected embryos (tables 1 and 2). Only 4 exceptions occurred in the data on decreased body weights. Two of these were thiouracil-injected embryos and only one chick was concerned in each instance. In the other two which involved thiourea-injected animals, the data were taken on the first (15th) or second (20th) day after the injection which may have given too short a period of exposure to the drug. It was interesting that in the former only a small increase in average thyroid weight had occurred and in the latter it had decreased (table 3). The fact that higher percentage decreases in average body weight usually occurred in chicks dissected on the 21st and 22nd days following an injection on the 8th than on the 14th suggested that the longer exposure was more effective. But prior to these days, the data were not con-

sistent with this conclusion. Moreover, even on the 21st day, the percentage decreases for chicks injected with thiourea on the 8th and 14th, or 8th, 14th and 18th days, were lower than that for those whose treatment began on the 14th. Neither did two or three injections (4.0 or 6.0 mg of thiourea) compared with one (2.0 mg) cause a relatively greater decrease in body weight. In fact where data were available for comparison as on the 18th, 21st and 22nd days, the decreases with one exception were less. In thiouracil-injected chicks, 2.0 mg of the drug were more effective than 1.0 mg by the 22nd day, although on the 18th, the reverse was true. The data on the length of the tibiotarsus were more consistent, especially after the 15th day, but for both leg length and body weight, more data should be gathered to provide evidence as to the effect of exposure and dosage on growth. Fugo's hypophysectomized chicks were of smaller size than the controls but he attributed this deficiency to lack of pituitary growth factor rather than to hypothyroidism.

Retardation of growth was not mentioned in either of two reports that were concerned with exposure of chick embryos to antithyroid compounds. The first study was that of Andrews and Schnetzler ('45) who raised chicks from eggs laid by hens given thiouracil. Although they could demonstrate the presence of the drug in the yolk of such eggs, they did not describe any modification in development of the embryos. The second paper, that of Grossowicz ('46) already mentioned, emphasized retardation of hatching and lack of retraction of yolk sac. However, treatment of chicks beginning immediately after hatching or even of partially or fully grown fowl has in general resulted in lessened general growth or a slowing of growth of particular parts such as bones, or regenerating feathers (Astwood, Bissell and Hughes, '44; Briggs and Lillie, '46; D'Angelo et al., '47; Domm and Blivaiss, '44, '48; Glazener and Jull, '46; Juhn, '44, '46; Kempster and Turner, '45). There have been a few reports of no effect or even increased growth (Astwood, Bissell and Hughes, '44; Schnetzler, Andrews and Hauge, '45).

As is suggested by Charipper and Gordon ('47, p. 286), "these discrepancies may be due perhaps to the differences in the ages of the birds and lengths of treatment employed." It is also true that judgments based on body weight as a criterion of growth may be misleading since at least in older birds fat deposition occurs after the administration of antithyroid drugs (Schultze and Turner, '44; Andrews and Schnetzler, '46).

In mammals many instances of retarded growth after administration of antithyroid drugs have been reported (Astwood et al., '43; Christensen, '45; Dempsey and Astwood, '43; Fitzhugh and Nelson, '47; Goldsmith, Gordon and Charipper, '45; Gordon, Goldsmith and Charipper, '45; Higgins, '45; Hughes, '44; Leathem, '45a and b; Mackenzie and Mackenzie, '43; Williams, Weinglass, Bissell and Peters, '44). Only dogs and monkeys seem to be refractory to treatment with these drugs (Aranow, Engle and Sperry, '46; Danowski, Man and Winkler, '46; Mayer, '47). Especially pertinent to the present experiments are the cretinous changes which have occurred in rats exposed to antithyroid drugs during gestation or immediately after birth (Hughes, '44; Goldsmith, Gordon and Charipper, '44, '45; Parmer, '47). It is also interesting that these drugs inhibit metamorphosis in anuran and urodele tadpoles (Gordon, Goldsmith and Charipper, '43, '45a; Hughes and Astwood, '44; Gasche, '46; Gasche and Druey, '46; Lynn and DeMarie, '46; Lynn, '47, '48; Thomas, '47; Adams and Craig, '49a) but in some instances general body growth in the anurans has continued although limb growth has not (Gordon, Goldsmith and Charipper, '45a; Adams and Craig, '49a).

The third effect of the antithyroid compounds on chick embryos exposed during incubation was the enlargement of the thyroid gland which occurred consistently (one exception) in the entire series of experiments (table 3). This type of response has been reported by many investigators, both in birds (Andrews and Schnetzler, '46; Astwood, Bissell and Hughes, '44; Domm and Blivaiss, '44, '48; Larson, Keating,

Peacock and Rawson, '45; Mixner, Reineke and Turner, '44; Schultze and Turner, '44, '45; Turner, '46a and b; VanderLaan and Bissell, '46a and b) and mammals (Astwood et al., '43; Mackenzie and Mackenzie, '43; and others: see review of Charipper and Gordon, '47).⁴ The average percentage increases in the chick embryos ranged from 5.2 to 347.8% but in 29 of the 45 groups, they exceeded 50% and in 12 of these the gland had more than doubled in size (table 3). In certain individuals, the thyroids were even 4 or 5 times the weight of those in their controls. Much greater increases in thyroid size have been reported: up to 45 times in chicks given thiouracil for 10 weeks (Astwood, Bissell and Hughes, '44); up to 15 times in a Barred Rock-New Hampshire Red cross fed a ration which included 0.1% thiourea for one to 20 weeks after hatching (D'Angelo et al., '47) and up to 7.5 times in chicks fed 0.1% thiouracil for 10 days (Dvoskin, '47). It should be noted that in most of these cases, both the dosages and the duration of treatment were much greater than in the present study.⁴ In young rats a three-fold increase followed administration of thiouracil (Astwood and Bissell, '44). Data in the present experiments were not conclusive as to the possibly greater effect of double or triple doses compared with a single dose or as to the length of exposure to the drug on increasing the thyroid weights. Besides the weight increases, the gland showed the typical hyperemia, hyperplasia (mitoses were abundant: cf. Astwood et al., '43; Paschkis et al., '45), heightened follicular epithelium (doubled or nearly doubled heights were common, cf. Dvoskin, '47), vacuolization and release of colloid described by other investigators (see review by Charipper and Gordon, '47).⁵ Some retardation in differentiation was observed in glands of chicks dissected on the 10th, 11th and 12th day, following an injection

⁴ It is possible that thyroid responses may differ in the two sexes (Mixner et al., '44) but numbers of chicks were too small to warrant any consideration of this factor.

⁵ Since these experiments were completed, hyperplasia and hypertrophy of the thyroid glands in lizards (*Anolis carolinensis*) have been secured with thiourea and thiouracil. See Adams and Craig, *Anat. Rec.*, 103: 565, 1949.

on the 8th. This may be compared with the atrophic condition described for thyroids of anuran tadpoles following immersion in thiourea (Gordon et al., '43) and would suggest that the gland in a relatively undifferentiated state may react somewhat differently from the differentiated one. As in the case of the thyroid weights, the data did not permit any conclusion as to the greater efficacy of long versus short exposure or double or triple doses versus a single one (table 4), although there appeared to be a tendency of the longer exposure to produce higher cells and higher percentage increases. The greatest exhaustion of colloid occurred in chicks given three injections of thiourea (8th, 14th, 18th days) and autopsied on the 21st day. It was apparent throughout the experiments that thiouracil was not as effective an antithyroid agent as thiourea. This was unexpected since the latter drug has been reported to be only 1/3 to 1/10th as potent as the former (Astwood, '45; VanderLaan and Bissell, '46a). However, the thiouracil did not go into solution readily and so absorption may have been less. Also, the number of chicks treated was much fewer, since in each group usually only one chick was injected with thiouracil compared with two or three given thiourea.

All of the results secured in the present experiments fit the interpretation that the antithyroid drugs have produced a state of hypothyroidism in the chick embryos, as in other animals, due mainly to the inability of the thyroid to synthesize thyroid hormone and that the lowered thyroid hormone level has brought about a greater production of thyrotrophin by the pars anterior and so caused the hyperplasia and hypertrophy of the thyroid (Astwood, '43, '45, '49; Charipper and Gordon, '47; Mackenzie, '47; VanderLaan and Bissell, '46a and b). The hypothyroid condition was revealed in retardation of hatching, lack of yolk sac retraction, decreased body and leg growth. Thus the embryo chick has proved to be very responsive to a lack of thyroid hormone during incubation, just as it has been shown to be responsive to an excess (Willier, '24; Woodside, '37).

SUMMARY AND CONCLUSIONS

In experiments on White Plymouth Rock chick embryos, doses of 0.5 cm³ of a solution of 0.4% thiourea or of 0.1% or 0.2% thiouracil were injected on the (1) 8th, (2) 14th, (3) 8th and 14th, (4) 8th, 14th and 18th, and (5) 18th days of incubation. Sets of chicks receiving injections on the 8th or 14th days were dissected every day thereafter (with the exception of the 23rd) until the 24th. The other groups were dissected at various times (table 1). Controls received injections of distilled water or were not injected.

The main effects of the injections of the antithyroid drugs were (1) retardation of hatching in those eggs incubated to the 21st through the 24th days and lack of retraction of the yolk sacs; (2) decreased growth as judged by lowered body weights and shortened limbs; and (3) enlargement of the thyroids with typical hyperemia, hyperplasia, and hypertrophy, a marked increase in height of follicular epithelium, vacuolization of colloid and colloid release. There was some indication that the longer exposure to the drugs, i.e., from the 8th day, produced a greater effect but data on this point and on the possibly greater effectiveness of double and triple doses compared with single ones, were not wholly conclusive. Thiourea was more effective than thiouracil.

The results of the experiments fall in line with the interpretation that antithyroid drugs prevented the synthesis of thyroid hormone and so produced a state of hypothyroidism in the chick embryos.

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PLATES

PLATE 1

EXPLANATION OF FIGURES

1 Normal chick dissected on the 21st day of incubation. Body weight without yolk sac, 29.7 gm. Note thyroid glands at base of carotid arteries. $\times 2/3$.

2 Thiourea-injected chick dissected on the 21st day of incubation. Body weight without yolk sac, 17.9 gm. Chick received 0.5 cm³ of 0.4% thiourea in its yolk sac on the 8th, 14th and 18th days of incubation. Note smaller size of chick and hypertrophied thyroid glands. $\times 2/3$.

3 and 4 Left legs of normal and thiourea-injected chick embryos killed on the 21st day of incubation, fixed in 10% formalin and stained in alizarin red S. $\times 5/6$.

3 N, leg of normal chick; length of tibiotarsus 21.3 mm; E, leg of chick given 0.5 cm³ of 0.4% thiourea in yolk sac on the 8th day of incubation; length of tibiotarsus, 17.6 mm. Note also smaller size of tarso-metatarsus and phalanges.

4 N, leg of normal chick; length of tibiotarsus, 20.6 mm; E, leg of chick given 0.5 cm³ of 0.4% thiourea in yolk sac on the 8th, 14th and 18th days of incubation; length of tibiotarsus, 17.5 mm. Note also smaller size of tarso-metatarsus and phalanges.

5 Section of thyroid of normal chick on the 14th day of incubation. Note homogeneous colloid and low cuboidal follicular epithelium. $\times 153$.

6 Section of thyroid of chick given 0.5 cm³ of 0.4% thiourea in yolk sac on the 8th day of incubation and killed on the 14th day. Note increased height of epithelium and decreased colloid compared with normal gland (fig. 5). Mitoses are numerous. $\times 153$.

7 Section of thyroid of normal chick killed on the 21st day of incubation. Note flat to low cuboidal epithelium and homogeneous colloid. $\times 153$.

8 Section of thyroid of chick given 0.5 cm³ of 0.4% thiourea in yolk sac on the 8th, 14th and 18th days of incubation and killed on the 21st day. Note heightened epithelium and loss of colloid compared with normal gland (fig. 7). $\times 153$.

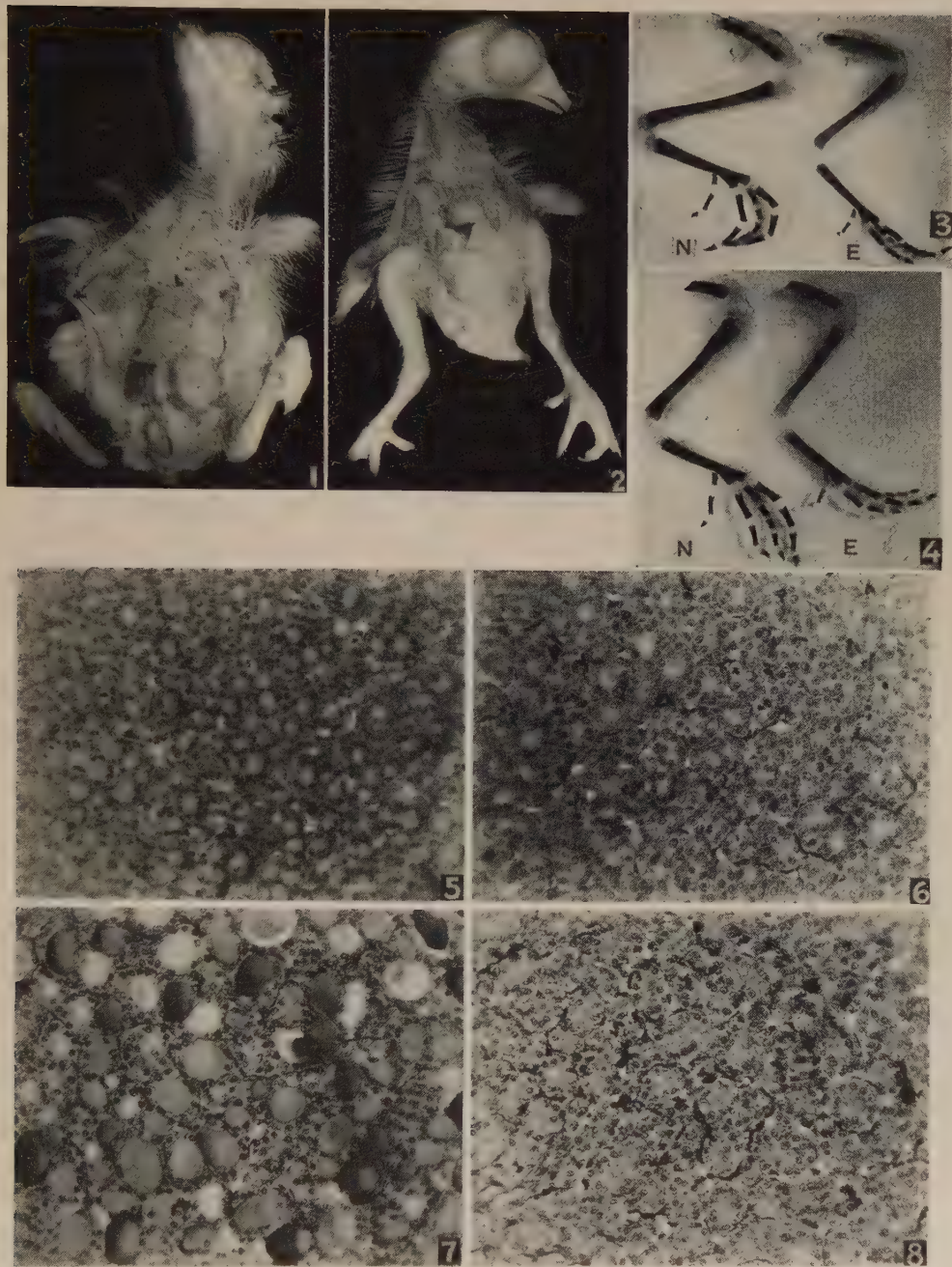


PLATE 2

EXPLANATION OF FIGURES

9 Section of thyroid of normal chick on 21st day of incubation. Note squamous epithelium and homogeneous colloid. $\times 180$.

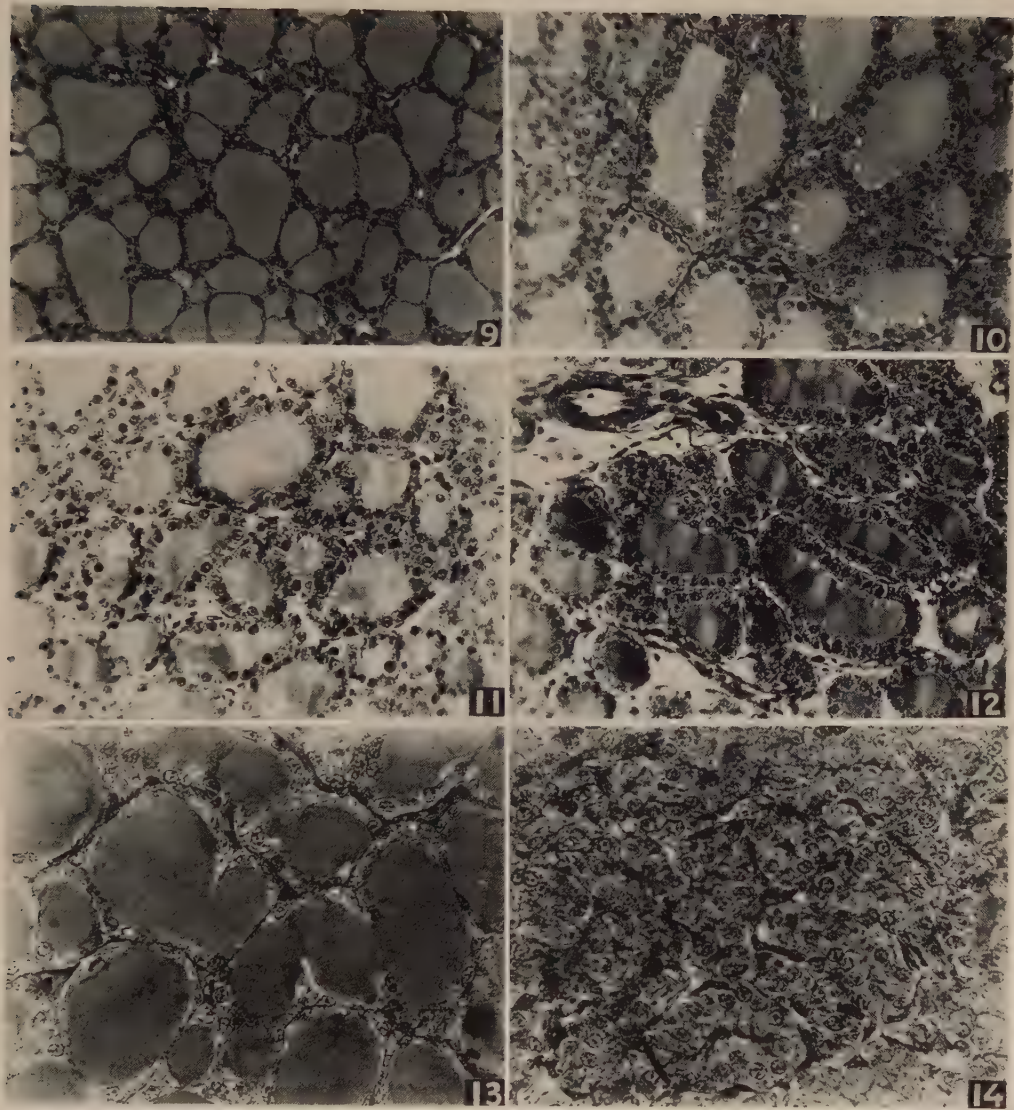
10 Section of thyroid of chick given 0.5 cm^3 of 0.4% thiourea in yolk sac on the 8th day of incubation and killed on the 21st day. Note increased height of epithelium, vacuolation of colloid, and numerous mitoses. Compare with figure 9. $\times 180$.

11 Section of thyroid of chick given 0.5 cm^3 of 0.4% thiourea in yolk sac on the 14th day of incubation and killed on the 21st day. Note increased height of epithelium and vacuolated colloid. Compare with figures 9 and 10. $\times 180$.

12 Section of thyroid of chick given 0.5 cm^3 of 0.4% thiourea in yolk sac on the 8th and 14th days of incubation and killed on the 22nd day. Increased height of epithelium and vacuolation of colloid compared with the normal gland (fig. 9) are apparent. $\times 180$.

13 Section of thyroid of normal chick killed on the 21st day of incubation. Follicles are large and some are fusing. Epithelium is squamous or low cuboidal and colloid is homogeneous. Compare figure 9. $\times 330$.

14 Section of thyroid of chick given 0.5 cm^3 of 0.4% thiourea in yolk sac on the 8th, 14th and 18th days of incubation and killed on the 21st day. Note high columnar epithelium and loss of colloid. Compare with figures 13 and 8. $\times 330$.



RESPONSE BY THE RAT THYRO-PARATHYROIDECTOMIZED AT BIRTH TO GROWTH HORMONE AND TO THYROXIN GIVEN SEPARATELY OR IN COMBINATION¹

I. GENERAL GROWTH AND ORGAN CHANGES

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EIGHT FIGURES

INTRODUCTION

Rats thyro-parathyroidectomized at birth show marked retardation in growth and in maturation (Salmon, '38; Scow and Simpson, '45). Administration of purified growth hormone to such animals resulted in an increase in the rate of growth, both in body weight and length, without an increase in the rate of maturation (Scow and Marx, '45; Becks, Simpson, Scow, Asling and Evans, '48). These experiments suggest that the hypophyseal growth hormone is essential for growth, i.e., increased body dimensions, but it is not able to stimulate the process of differentiation or maturation in the thyro-parathyroidectomized young animal. It seems that the thyroid hormone is necessary for the latter process. To test this hypothesis pure growth hormone and thyroxin were given separately and in combination to rats thyro-parathyroidectomized at birth. This report describes the difference in growth and differentiation which resulted.

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Method

Thyro-parathyroidectomy was performed on approximately 300 one-day old rats of both sexes of the Long-Evans strain. The operative technique and anesthesia which were employed have been previously described (Scow and Simpson, '45; Scow, '49). The technique was improved by using an electro-cautery to divide the superior thyroid arteries. Postoperative infections were suppressed by sprinkling a small amount of sulfathiazole powder in the subcutaneous layer of the incision at the time of closure.

Following operation under refrigeration anesthesia, the animals were warmed before being returned to the mothers. They were weaned at 30 days of age. A slightly modified McCollum's Diet I was available before and after weaning, both in the dry form and mixed with water. All the animals apparently preferred the moist diet.

Although the rats were kept in a room maintained at a temperature of 75°F., it became obvious during the experiment that further increase of the environmental temperature was necessary for these rats except when thyroxin was being administered. An incandescent lamp was placed against one wall of each cage. The rats would remain near or in contact with this wall, and the benefit was shown by increased activity and appetite, greater gains in body weight, and improvement in general appearance and health.

The injection period began when the rats were 31 days of age, and continued for 30 days. The rats were weighed at 5 day intervals during this period. Roentgenograms of each animal were made under ether anaesthesia at 30 days of age and again at 61 days of age. In addition, selected rats were anaesthetized and roentgenographed during the injection period at 45 days of age.

From the 300 rats operated upon, 60 were selected at 30 days of age as probably completely thyro-parathyroidectomized. The criteria for this selection have been previously described (Scow and Simpson, '45), and consisted of retardation in development of hair, ears, eyes and genitalia, stunted growth

(weight and length) and retardation of the skeletal development as determined roentgenographically. This last was the most critical criterion available during life, since it has been found that when the skeletal development of such rats at 30 days of age corresponds to that of a normal 15-day old rat, serial sections and radioautographs of the neck region after autopsy showed that in approximately 50% of such rats thyroid ablation was complete.

The rats presumptively shown to be completely thyroparathyroidectomized were divided into 4 groups of 15 animals each. The first group received injections of anterior hypophyseal growth hormone in a daily dose of 0.5 mg for 30 days. Each dose contained 25 rat units of growth hormone, as assayed in hypophysectomized, immature female rats (Li, Evans and Simpson, '45). The growth hormone was pure, judged by physicochemical criteria, and gave no evidence of the presence of other known pituitary hormones when injected in high doses. Another group of rats received crystalline thyroxin (Squibb) injected subcutaneously in a daily dose of 2.5 μ g. Like the growth hormone, this was given 7 days a week for 30 days. The third group of rats received injections of both growth hormone and thyroxin in the doses specified. A 4th group received no injections. In addition, two groups of unoperated, uninjected rats were sacrificed, one at 61 to 64 days of age, and the other at 18 days of age. The former corresponded in chronological age to the experimental animals at the time of autopsy, while the latter corresponded closely in size and degree of skeletal immaturity to the uninjected controls at the time of autopsy.

At the conclusion of the experiment the completeness of the operation was verified by the radioautographic technique of Reinhardt ('42), employing injections of radioactive iodine (I^{131}) and block dissection of the tissues in the laryngeal and superior mediastinal regions. It was more difficult to interpret the radioautograph in the animals which had received thyroxin, possibly because the hormone suppressed the function of any remaining thyroid fragments, reduced the uptake of the iodine tracer, and thus prevented the production of a

radioautographic shadow. Only those thyroxin-injected animals in which no shadow was present were considered completely thyro-parathyroidectomized, although in the other groups of rats, a shadow size of less than 1.0 sq. mm was considered as negative.³

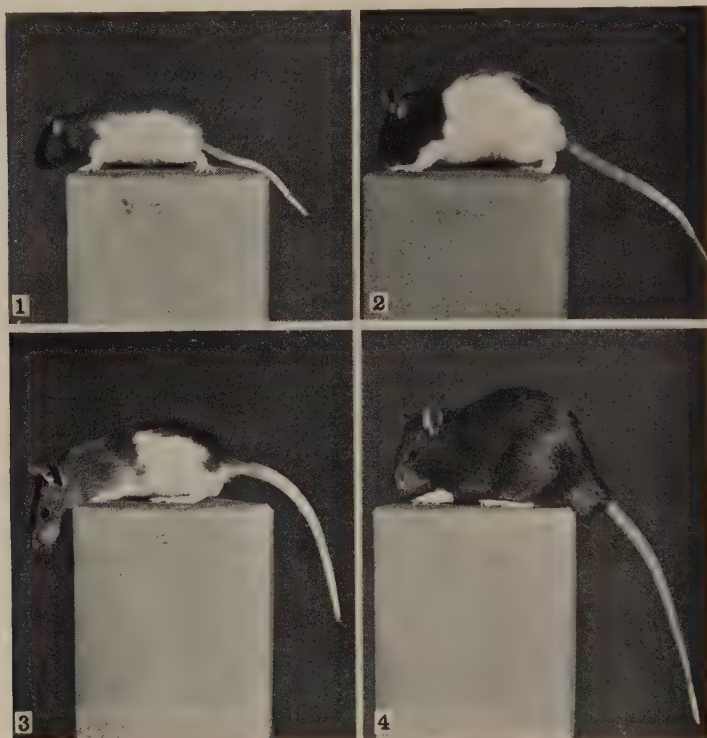


Fig. 1 Rat (female) thyro-parathyroidectomized on the first day of life. Uninjected control, at 61 days of age.

Fig. 2 Rat (female) thyro-parathyroidectomized on the first day of life and injected with 0.5 mg of pure pituitary growth hormone daily from 31 to 61 days of age.

Fig. 3 Rat (female) thyro-parathyroidectomized on the first day of life and injected with 0.0025 mg of thyroxin daily from 31 to 60 days of age.

Fig. 4 Normal (male) rat. Uninjected control, at 61 days of age.

³ Four of the 6 uninjected animals had shadows between 1 and 8 mm² in area but were considered as completely thyro-parathyroidectomized because the shadow was very faint and the skeletal age of each rat was no greater than that of completely thyro-parathyroidectomized rats.

RESULTS

As previously reported, the uninjected controls retained an infantile appearance. They were sluggish and inactive throughout the period of the experiment. The hair remained short, soft, and fine as in immature rats (fig. 1). They grew

TABLE 1

Growth in body weight and length of rats thyro-parathyroidectomized at birth. Rats injected for the 30-day period between 31 and 61 days of age with growth hormone and/or thyroxin, together with their athyroid and normal controls

TREATMENT	SEX ¹	NO. OF RATS	AVERAGE WEIGHT			AVERAGE LENGTH		
			Initial	Final	Gain	Initial	Final	Gain
			gm	gm	gm	cm	cm	cm
Growth hormone	♂ ♀	6	39	74	35	16.1	22.1	6.0
Thyroxin	♂	7	40	103	63	16.6	28.0	11.4
	♀	4	34	102	68	15.4	27.5	12.1
Thyroxin + growth hormone	♂	2	39	139	100	16.9	29.1	12.2
	♀	8	38	111	73	16.0	29.3	12.6
Uninjected controls	♂ ♀	6	34	49	16	15.5	18.9	3.4
18-day normal controls	♂	6	..	37	..	...	17.1	..
	♀	6	..	46	..	...	...	...
61-day normal controls	♂	9	92	217	125	26.1	34.2	8.1
	♀	11	86	160	74	24.8	32.4	7.6

¹ See text and footnote.

very slowly, as shown in table 1 and the body weight curve in figure 5. Their average weight gain during the 30-day period in which the other groups of animals were receiving injections was only 16 gm, although the weight gain of normal rats during the same period averaged 125 gm for males and 74 gm for females.⁴ In body length these rats grew 3.4

⁴ The genital organs failed to become functional in the uninjected controls and no sex differences were developed. Males and females have therefore been considered together. The same was true for the rats injected with growth hormone. Some gonadal stimulation occurred, however, in all rats injected with thyroxin, and males and females have therefore been considered separately.

cm during 30 days, in which time normal rats showed an average growth of 8.1 and 7.6 cm for males and females respectively.

The injections of growth hormone did not alter the infantile characteristics of the animals, nor did the activity of these injected rats increase. The hair remained immature as in the controls (fig. 2). Their body weight gain during the injection period was 35 gm, double that of the controls. In length they gained 6 cm, almost double the gain of the controls.

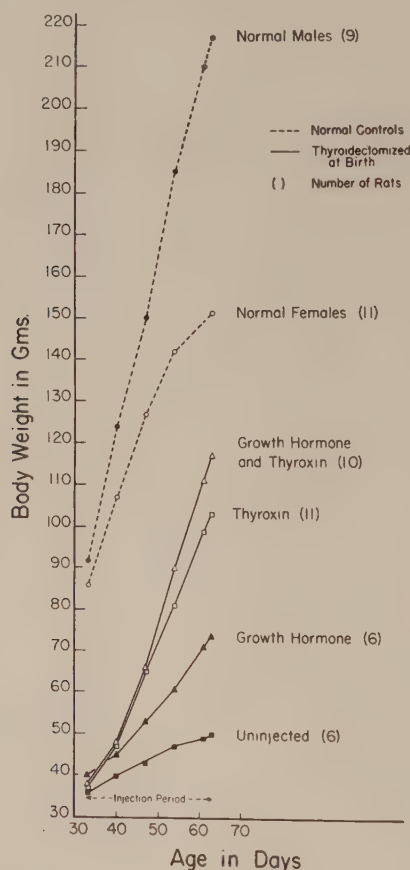


Fig. 5 Body weight gains of rats thyro-parathyroidectomized at birth and injected for the 30 day period between 31 and 61 days of age with growth hormone, thyroxin, or the combination; comparison with normal and thyro-parathyroidectomized controls.

Injection of thyroxin resulted in a marked acceleration of development. The rats were more active and alert. Changes could be seen in the development of the peltage, ears, and external genitalia within a few days after treatment began. One of the more conspicuous responses was the rapid change to the adult type of peltage, with long, stiff, coarse hairs (fig. 3; compare with the normal rat in fig. 4). Body weight gain during the injection period was 4 times that of the controls, being 63 and 68 gm for males and females respectively. Figure 5 shows that this rate of gain virtually paralleled that of normal rats. The average gain in length was 11–12 cm, also almost 4 times that of the controls.⁵

When growth hormone and thyroxin were given together, the animals showed the same rapid development as with thyroxin alone. The body weight and length gain were not significantly greater than with thyroxin alone.

In order to analyze further the skeletal growth under these different experimental conditions, various portions of the appendicular and axial skeleton were measured roentgenographically. The regions chosen for analysis were the tibia, the 6 lumbar vertebrae, the first 10 caudal vertebrae and the next 10 (11th to 20th caudal vertebrae).⁵ In addition, the first 4 caudal vertebrae were measured as a separate group, to determine whether their growth corresponded more closely to that of the vertebrae of the torso (represented by the lumbar region) than to that of the tail.

In order to interpret these data it was necessary to compare the growth of these bones with the growth of those of normal rats. Figure 6 shows the growth of the tibial diaphysis and different vertebral segments in normal rats during the period

⁵ Earlier roentgenographic studies have shown that measurement of the length of the entire roentgenographic shadow of the tibia does not yield comparable results in the various groups since the shadow of the epiphyseal ossification centers is faint or absent when their development has been retarded, in contrast to the sharp shadow seen when development has been accelerated by the treatment. Therefore only the diaphysis of the tibia was measured, since its shadow is sharp in all groups. Similar reasons determined the measuring methods used on the vertebral column. Each such measurement was extended to the midpoint of the intervertebral disc at either end of the group of 10 vertebrae studied, and included also the intervening intervertebral discs.

of the experiment. It shows the rapid growth in all these dimensions which takes place between 15 and 30 days of age and the somewhat slower rate at which they grow between 30 and 60 days of age. It shows further that the first to 10th, and 11th to 20th caudal vertebral regions have a parallel growth rate during this period. Finally, it illustrates that, as suspected, the proximal (first to 4th) caudal vertebrae have a growth rate more like that of the lumbar vertebrae than of the more distal caudal segments.

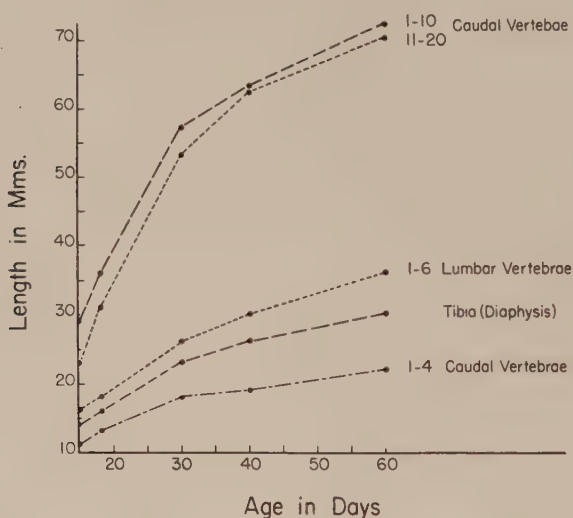


Fig. 6 Growth of the tibial diaphysis and the different vertebral segments in normal rats during the period of the experiment (from birth to 61 days of age).

Figure 7 shows the dimensions of the tibial diaphysis, the first to 6th lumbar vertebrae, and the first to 10th caudal vertebrae in the 4 groups of thyro-parathyroidectomized rats at the beginning and end of the injection period. The corresponding normal curves are given for comparison. The graph shows the slow skeletal growth in the untreated controls, and the improvement which resulted from growth hormone injections. Thyroxin injections resulted in more marked growth of these bones, approaching or even equalling the early

rapid growth of normal rats. The growth resulting from injection of thyroxin and growth hormone together did not significantly exceed that with thyroxin alone.

Figure 8 is a graphic representation of the average lengths at autopsy of the caudal vertebral segments (first to 4th, 5th to 10th, and 11th to 20th) in the 4 groups of thyro-parathyroidectomized rats. They are to be compared not only with

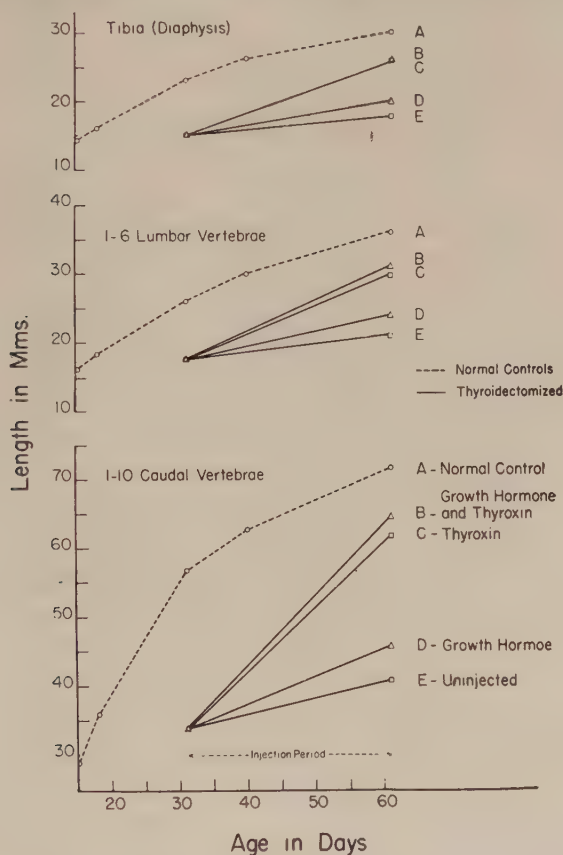


Fig. 7 Dimensions of the tibial diaphysis, the first to 6th lumbar vertebrae and the first to 10th caudal vertebrae, in the 4 groups of thyro-parathyroidectomized rats at the beginning and end of the 30 day injection period (31 and 61 days of age). The corresponding normal curves, taken from figure 6, are shown for comparison. Key: A, Normal; B, Thyroxin and growth hormone; C, Thyroxin; D, Growth hormone; E, Uninjected control.

the lengths of the same segments in the thyro-parathyroidectomized rats at the onset of injections but also with their lengths in normal rats of ages represented in the experiment. It may be seen that at the onset of the injection period, at 30 days of age, these dimensions in the thyro-parathyroidectomized rats fell between those of normal rats at 15 and 18

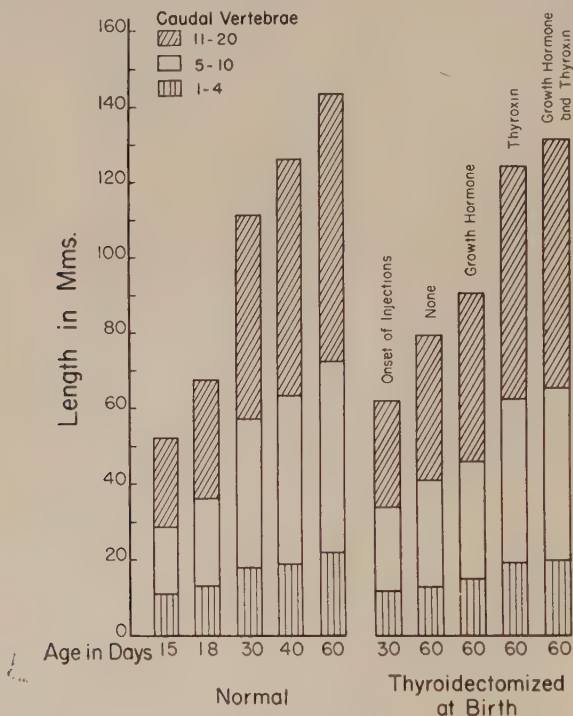


Fig. 8 Graphical representation of the differential growth of various segments of the caudal vertebrae in thyro-parathyroidectomized rats, treated and untreated, as compared with normal controls.

days of age. At the end of the experimental period, 61 days of age, the uninjected controls had grown slightly, so that these segments exceeded the length normal at 18 days. The thyro-parathyroidectomized rats injected with growth hormone showed greater growth, and had dimensions midway between the normal 18- and 30-day measurements. The rats

TABLE 2

Criteria for roentgenographic determination of skeletal age in the rat

OSSIFICATION CENTER	CHRONOLOGICAL AGE IN DAYS ¹							
	12	15	18	21	24	30	40	56
<i>Humerus</i>								
Head	X							
Capitulum	X							
Medial epicondyle				X				
<i>Radius</i>								
Head	?	X						
Distal epiphysis	X							
<i>Ulna</i>								
Olecranon	X							
Distal epiphysis	X							
<i>Carpals</i> ²	6	8						
<i>Metacarpals</i> , epiphysis	X							
<i>Phalanges</i> (proximal)								
Epiphysis		X						
<i>Femur</i>								
Greater trochanter					X			
Distal epiphysis	X							
<i>Patella</i>							X	
<i>Tibia</i>								
Proximal epiphysis	X							
Distal epiphysis	X							
<i>Fibula</i> , distal epiphysis	X							
<i>Tarsals</i> ²	4	5-6	6-7					
<i>Metatarsals</i> , epiphysis		X						
<i>Phalanges</i>								
Proximal, epiphysis			X					
Middle, epiphysis				X				
<i>Ribs</i> , ventral extension ³							X	
<i>Caudal vertebrae</i> ⁴								
Epiphyseal centers present			5	10-11	15-16	21-22	26-28	
Epiphyseal centers fused					1-2	6-7	11-12	20-21

¹ The symbol X indicates the earliest age studied at which the ossification center was clearly visible on roentgenograms.

² The number of carpal or tarsal ossification centers visible.

³ Age at which ossification has extended to the ventral aspect of the body.

⁴ Number of caudal vertebrae having the described attribute, counting from the first.

injected with thyroxin equalled 40-day old normals in caudal segment lengths. The bones of rats receiving both growth hormone and thyroxin slightly exceeded those receiving thyroxin alone, but did not equal the dimensions of normal 60-day old rats, their chronologic equals. In spite of the differences in growth of the tail of the thyro-parathyroidectomized rats, untreated and treated, the different segments of the tail maintained proportionality.

TABLE 3
Skeletal ages of rats thyro-parathyroidectomized at birth

TREATMENT	NO. OF RATS	SKELETAL AGE AT THE FOLLOWING CHRONOLOGICAL AGES (DAYS)		
		31	45 ¹	61
Growth hormone	6	15	15 (1)	18
Thyroxin	11	15	30 (4)	>40<56
Thyroxin + growth hormone	10	15	30 (3)	>40<56
Uninjected control	6	15		18

¹ The number in parenthesis indicates the number of rats whose skeletal age was determined at this chronologic age. They were anaesthetized and roentgenographed at the mid-point of the injection period.

The effect of these treatments on skeletal differentiation was studied by determining the time of appearance of representative ossification centers and of disappearance of epiphyseal lines as shown in roentgenograms. Table 2 shows the data, obtained from normal rats of the Long-Evans strain, which served as criteria for determining the skeletal age of rats in the various experimental groups.

The skeletal ages attained by the various groups of thyro-parathyroidectomized rats, treated and untreated, are given in table 3. The previous observation was confirmed that rats thyro-parathyroidectomized at birth have a skeletal age of 15 days when chronologically 30 days of age, and that during the succeeding 30-day period they advance in skeletal differentiation by three days.⁶

⁶ See footnote 3, page 448.

Injection of growth hormone did not stimulate skeletal differentiation, although it will be remembered that the skeletal dimensions did increase. Like the uninjected controls, the rats so treated advanced in skeletal age only three days during the 30 days of injections.

Thyroxin, alone or with growth hormone, did accelerate skeletal differentiation. During the first 15 days of injections, the skeletons of these rats developed at the rate at which a normal 15-day old rat differentiates. In the next 15 days they attained a skeletal age beyond 40 days, but did not reach a skeletal age of 61 days (their chronological age).⁷ The skeletal age of the normal controls confirmed earlier determination of bone age at their respective ages.

Table 4 gives the weights of the viscera, including the liver, kidneys, gastrointestinal tract, spleen, lymph nodes (mesenteric), a representative muscle mass (gastrocnemius and soleus), brain, and eyeball. It shows that all of these viscera grew in all treated groups of rats. Their growth was either at the same rate or faster than that of the body, with rare exceptions. The brain and eyeball grew only slightly, and therefore decreased in proportion to body weight.

Table 5 gives the weights of the reproductive and endocrine viscera, including one ovary, uterus (with one oviduct), both testes, prostate, seminal vesicles, pituitary, and both adrenals.

The ovaries of the uninjected controls were extremely small but usually contained several small to medium follicles, mostly in atresia. The interstitial tissue was atrophic. The ovaries of rats injected with growth hormone were like those of controls. Thyroxin caused some enlargement of the ovaries. The follicles were only slightly increased in size but the interstitial cells were repaired. The combination of the two hormones had the same effects on the ovaries as thyroxin alone. The uteri of all injected groups were somewhat larger than

⁷ The normal equivalent attained by these two groups of rats cannot be stated exactly, since it is difficult to determine roentgenographically the differences between the skeletal development of rats 40 and 60 days of age.

TABLE 4
Average weights of viscera of rats thyro-parathyroidectomized at birth

TREATMENT	SEX ¹	NO OF RATS	B.W. AT AUTOPSY	LIVER	KIDNEYS	G.I. TRACT	SPLEEN	LYMPH NODES	MUSCLE	BRAIN	EYEBALL
			gm	gm	gm	gm	gm	mg	gm	gm	mg
Growth hormone	♂ ♀	6	74	3.0	0.72	4.6	6.2	132	0.31	1.38	97
			%B.W.:	4.1	0.97	5.7	0.82	0.178	0.42	1.87	0.131
Thyroxin	♂ ♀	7	103	4.7	1.16	5.7	0.92	170	0.48	1.48	106
			%B.W.:	4.5	1.12	5.3	0.89	0.165	0.47	1.44	0.103
Thyroxin + growth hormone	♂ ♀	4	102	4.4	0.99	5.3	0.86	186	0.54	1.44	97
			%B.W.:	4.3	0.97	5.2	0.84	0.183	0.53	1.41	0.095
Thyroxin + growth hormone	♂ ♀	2	139	5.7	1.38	6.8	0.89	176	0.72	1.51	104
			%B.W.:	4.1	0.99	4.9	0.64	0.127	0.52	1.09	0.075
Uninjected control	♂ ♀	8	111	4.9	1.25	5.7	0.77	168	0.56	1.48	105
			%B.W.:	4.4	1.12	5.1	0.69	0.150	0.50	1.34	0.094
18-day normal control	♂ ♀	6	49	2.0	0.51	3.3	0.36	84	0.19	1.19	92
			%B.W.:	3.7	1.02	6.7	0.51	0.170	0.39	2.40	0.188
61-day normal control	♂ ♀	6	37	1.5	0.50	1.9	0.15	27	0.13	1.29	62
			%B.W.:	4.1	1.35	5.1	0.40	0.073	0.35	3.50	0.168
	♂ ♀	6	46	2.1	0.59	2.2	0.25	38	0.18	1.33	66
			%B.W.:	4.5	1.28	4.8	0.54	0.083	0.39	2.90	0.143
	♂ ♀	9	217	8.0	1.91	9.6	0.73	410	1.15	1.66	102
			%B.W.:	3.8	0.90	4.5	0.34	0.190	0.53	0.78	0.048
	♂ ♀	11	160	5.9	1.26	6.3	0.59	256	0.98	1.58	108
			%B.W.:	3.7	0.79	3.9	0.37	0.160	0.61	0.99	0.018

¹ See text and footnote, page 449.

TABLE 5
Average weights of reproductive and endocrine organs of rats thyro-parathyroidectomized at birth

TREATMENT	SEX ¹	NO. OF RATS	BODY WEIGHT, gm AT AUTOPSY	WEIGHTS OF REPRODUCTIVE ORGANS					WEIGHTS OF ENDOCRINE ORGANS		
				Ovary	Uterus	Testes	Prostate	Seminal vesicles			
				mg	mg	gm	mg	mg	Pituitary	Adrenals	mg
Growth hormone	♂ ♀	6	74	7.0	63.2	0.30	56.0	12.5	4.0	31	
Thyroxin	♂ ♀	7 4	103 102	9.7	82.3	0.88	91.3	29.0	4.0	32	
									4.0	28	
Growth hormone + thyroxin	♂ ♀	2 8	139 111	10.0	69.2	1.21	143.0	56.5	3.0	27	
									4.0	33	
Uninjected controls	♂ ♀	6	49	4.0	34.5	0.26	37.0	11.7	4.1	21	
18-day normal controls	♂ ♀	6 6	37 46	5.0	29.0	0.13	32.0	8.0	2.0	12	
									3.0	14	
61-day normal controls	♂ ♀	9 11	217 160	21.0	288.0	2.33	376.0	391.0	6.0	33	
									7.0	42	

¹ See text and footnote, page 449.

in controls but were still infantile structurally. The vagina also remained infantile in all treated animals.

The testes of the uninjected controls were very small. The tubules were small and plugged with desquamating spermatocytes of the first order. The interstitial cells resembled connective tissue cells, being less pyknotic than in hypophysectomized rats. Growth hormone injection caused no change in the testes. Injections of thyroxin, alone or with growth hormone, caused an increase in testis weight and development. The tubules were enlarged. Layers of healthy spermatocytes usually filled the lumen, spermatids being present in two of 7 rats. Interstitial tissue was epithelioid, though the cytoplasmic body was still small. The healthier condition of the interstitial tissue was reflected in the moderate enlargement of seminal vesicles and prostate in the groups receiving thyroxin.

The normal rats sacrificed at the termination of the experimental period, at 61 days of age, had fully developed testes containing sperm, and the interstitial cells were fully developed.

The adrenals were slightly increased in weight in all injected groups. The cortex was increased in width in groups receiving thyroxin and there was some reconstitution of the zona reticularis and a more uniform distribution of lipid.

DISCUSSION

The preceding results showed that injections of growth hormone in thyro-parathyroidectomized rats resulted in gain in body weight and skeletal dimensions, but did not affect the retarded maturation rate of the skeleton, skin, hair, and gonads. It has been suggested that the dwarfism of thyroidectomized rats is due to decreased output of growth hormone by the pituitary gland. According to this theory the mechanism of thyroid therapy is to restore, at least in part, the production or release of growth hormone by the pituitary. In this connection it should be noted that the dose of pituitary growth hormone injected in this experiment was less effec-

tive than thyroxin in stimulating growth of the thyro-parathyroidectomized dwarfs.⁸ This relative insensitivity to a pituitary hormone can best be attributed to the complete absence of the thyroid. It was noted that in incompletely thyro-parathyroidectomized rats minute traces of thyroid tissue, which in themselves were unable to produce any appreciable maintenance of growth, augmented the response to growth hormone injections. This might suggest that the greater sensitivity of the hypophysectomized rat to growth hormone may depend on the thyroid tissue present, which though histologically atrophic, may still form thyroid hormone in an amount sufficient to synergize the effect of exogenous growth hormone.

The growth which resulted from injections of thyroxin was not significantly augmented by the concurrent injection of growth hormone. It is postulated that the dosage of thyroxin used, which was well tolerated, not only sufficed to restore the tissue thyroxin level to normal, but also restored the production and release of growth hormone by the anterior pituitary. Animals growing as rapidly as these in response to endogenous growth hormone are relatively insensitive to supplies of exogenous growth hormone.

The hypogonadism observed in the thyro-parathyroidectomized rats may also have resulted from the deficient pituitary function, and the gonadal repair following thyroxin administration similarly may have been due to resumption of gonadotropic output by the pituitary. In incompletely thyro-parathyroidectomized rats it is noteworthy that when the persisting fragments of thyroid tissue were too small to support growth and somatic maturation, appreciable gonadal development was seen, indicating that lower levels of thyroid

⁸ However, it should be noted that the growth response of thyro-parathyroidectomized rats to growth hormone is much less than that of hypophysectomized rats; the dose used to produce an average daily weight gain of 1 gm in thyroidectomized rats was 20 to 25 times as great as that required to produce the same gain in rats hypophysectomized at 28 days of age (the standard test animal used in assay of the hormone).

hormone sufficed for production and release of pituitary gonadotropins than are necessary for growth hormone.

SUMMARY

Approximately 300 rats were thyro-parathyroidectomized on the day of birth. At 30 days of age thyroid ablation was judged to be complete in 60 of these rats on the basis of roentgenographic estimation of skeletal development (bone age equal to 15 days), lack of development of body form, ears, peltage, and external genitalia, and deficient body weight. They received injections of pituitary growth hormone and/or thyroxin from 31 to 61 days of age. At autopsy, completeness of operation was determined by radioautography, using I^{131} and block removal of neck and superior mediastinal tissue.

During the injection period untreated controls increased in body weight from 34 to 49 gm. Total body length increased 12% and bone age advanced three days (to 18 days).

The rats receiving thyroxin, alone or with growth hormone, tripled their body weight. Their body form, ears, peltage and genitalia showed marked development. Body length increased 17-18% and their bone age advanced to 40 days. The rats receiving growth hormone alone doubled their body weight. However, their body form, ears, peltage, and genitalia remained immature as in the controls. Their body length increased 14%. Their skeletal age, like that of the untreated rats, advanced only three days.

The non-endocrine viscera of injected animals maintained proportionality to body weight with the exception of brain and eyeball. Endocrine and reproductive viscera developed only in rats receiving thyroxin.

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RESPONSE BY THE RAT THYRO-PARATHYROIDECTOMIZED AT BIRTH TO GROWTH HORMONE AND TO THYROXIN GIVEN SEPARATELY OR IN COMBINATION

II. HISTOLOGICAL CHANGES IN THE PITUITARY ¹

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FIVE FIGURES

This report concerns the histology of the pituitaries of rats thyro-parathyroidectomized at birth and the "corrective" changes which ensued after thyroxin and growth hormone therapy. The general effects of the operation have been described by Scow, Simpson, Asling, Li and Evans, ('49) in a preceeding paper of this series. In brief, they reported marked retardation in the growth and skeletal maturation in the thyro-parathyroidectomized rats. Administration of growth hormone resulted in increased rate of growth, both in weight and skeletal dimensions, without increase in rate of skeletal maturation. Thyroxin in low doses caused a marked increase in body weight and length with increased rate of maturation. Body form, ears, peltage, and genitalia of these rats showed marked development, and their bone age had advanced rapidly. The combination of growth hormone with thyroxin had only slightly greater effects than thyroxin alone.

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There has been some lack of agreement in the literature regarding the details of the changes in the anterior pituitary in the rat after thyroid ablation. The following effects may be considered to be reasonably well established. Hohlweg and Junkman ('33), Severinghaus, Smelser and Clark ('34), Zeckwer and her coworkers ('35), Guyer and Claus ('37) and others have shown that the removal of the thyroid gland in mature and immature rats resulted in the following changes: pituitary weight was increased; acidophils were decreased in number; degranulation was widespread; basophils were markedly increased in number and were enlarged. The basophils invariably showed rapidly developing vacuolation. Such basophils are frequently referred to in the literature as "thyroidectomy cells."

As far as the effect of thyroid ablation on the pituitary of the newborn rat is concerned, Salmon in 1938 reported from preliminary observations of A. E. Severinghaus that the changes were similar to those described earlier by Severinghaus, Smelser and Clark ('34) as following thyroidectomy in older animals. Later Severinghaus ('42), referring to unpublished data, stated in regard to replacement therapy that in rats completely thyro-parathyroidectomized at birth, thyroid feeding beginning with the 40th postoperative day did not correct the thyroidectomy changes. The pituitaries contained only chromophobes and granulation did not reappear.

MATERIAL AND METHODS

As described by Scow *et al.*, ('49), approximately 300 rats were thyro-parathyroidectomized on the first day of life. Sixty were selected when 30 days of age as probably thyroidless. They were divided into 4 groups of 15 animals each. Beginning on the 31st day of life three groups were injected daily for a period of 30 days either with thyroxin (0.0025 mg), growth hormone (0.5 mg) or the combination. One group of operated animals was retained as untreated controls. Of the thyro-parathyroidectomized animals autopsied at 61 days of age 21 were selected as most certainly thyroidless for analysis

of the pituitary.³ Six of these rats had been treated with thyroxin; 5 with pure growth hormone; 5 with the combination; and 5 had not been injected. In addition, pituitaries from 6 normal rats of the same chronological age, 61–63 days, were used for comparison. Pituitaries were fixed in Zenker-formol, embedded in nitro-cellulose and sectioned at 4 μ . Modified Mallory-Azan method (Koneff, '38) was used as a routine staining procedure.

Percentages of the cell types were estimated by the method recommended by Rasmussen and Herrick ('22); not less than 2500 cells were counted in every gland studied. Acidophil and basophil cell-size was determined by planimetric measurements of the area occupied by the camera lucida outline of 100 cells.

RESULTS

The weights of pituitaries of the thyro-parathyroidectomized animals were much smaller than those of normal rats of the same chronological age. Neither therapy with thyroxin nor growth hormone, nor the combined therapy increased the pituitary weights. The proportion between pituitary weight and body weight, the relative weight of the pituitary, was high in the dwarfed, thyro-parathyroidectomized controls. The administration of thyroxin alone, or concurrently with growth hormone, decreased the relative weight of the gland whereas growth hormone injected alone had no significant effect (see table 1).

Histologically, the anterior pituitaries of the thyro-parathyroidectomized control rats, manifested well developed changes, comparable to those repeatedly described in the literature as characteristic of the pituitary of animals after a prolonged period following thyroid removal (fig. 1). Only small numbers of typical acidophils were found. Those still present in the gland were in various stages of degranulation. The acidophils were smaller than those in the normal pituitary (147 ± 2 versus 178 ± 2 , planimeter units, see table 2).

³ Methods used to determine completeness of thyroid ablation were discussed in a previous communication from this laboratory (Scow and Simpson, '45).

The basophils were markedly increased in number and changed morphologically. Many of them were at the maximum size ever attained by these cells as the result of accumulation in the cytoplasm of multiple vacuoles containing colloid material staining with aniline blue. Colorless vacuoles appeared within the dense blue hyaline material. Close scrutiny was required to detect unaltered, granular basophils. Here and there, large ballooned cells could be seen in which the cytoplasm was almost colorless and the small pyknotic nucleus had been displaced to the very periphery of the cell. Degenerating and fragmentary basophils, in which the outline of the individual cells was lost, were numerous. Shredded,

TABLE 1

Pituitary weights in rats thyro-parathyroidectomized at birth untreated and treated with growth hormone and/or thyroxin

TREATMENT	NO. OF RATS	WEIGHT MILLIGRAM		RELATION TO BODY WEIGHT. MG %	
		Mean	SE ¹	Mean	SE
Growth hormone	5	3.8 ± 0.2		5.2 ± 0.4	
Thyroxin	6	3.5 ± 0.2		3.4 ± 0.2	
Growth hormone and thyroxin	5	3.7 ± 0.2		3.2 ± 0.2	
Thyro-parathyroidectomized controls	5	3.5 ± 0.1		7.4 ± 0.7	
61 to 63 days old, normal controls	6	6.3 ± 0.2		3.5 ± 0.2	

$$^1 \text{SE (of mean)} = \frac{\text{SD}}{\sqrt{n-1}}$$

vacuolated basophilic masses were present, representing the remains of degenerating basophils. Droplets of hyaline material, remnants of nuclei and the negative image of the Golgi apparatus could at times be identified in such masses. Extreme changes leading to indefinite outlines of the basophils precluded counting or application of planimetric study.

Injections of growth hormone into thyro-parathyroidectomized rats did not alter markedly the picture created by the operation (fig. 2). The basophils appeared similar to those of thyro-parathyroidectomized control rats. Only a few

acidophils were identifiable, and they were even smaller in size than in the untreated controls and showed an even greater degree of degranulation.

Under the influence of thyroxin therapy the pituitaries of thyro-parathyroidectomized rats showed a remarkable degree of repair (fig. 3). The cells still differed from normal, however, in certain respects. The size of acidophils was still significantly smaller than normal (160 ± 2 versus 178 ± 2), even though they were granulated and constituted an almost normal percentage of these cells (see table 3).

TABLE 2
Size of acidophils and basophils in the pituitaries of rats in experimental and control groups

TREATMENT	NO. OF RATS	CELL SIZE IN PLANIMETER UNITS ¹			
		Acidophils		Basophils	
		Mean	SE ²	Mean	SE
Growth hormone	5	127 ± 4.0			
Thyroxin	6	160 ± 2.0		257 ± 7.0	
Growth hormone and thyroxin	5	147 ± 3.0		266 ± 6.0	
Thyro-parathyroidectomized controls	5	147 ± 2.0			
61 to 63 days old, normal controls	6	178 ± 2.0		302 ± 6.0	

¹ Area occupied by camera lucida tracings of 100 cells expressed in planimeter units.

$$^2 \text{SE (of mean)} = \frac{SD}{\sqrt{n-1}}$$

The basophil cells also benefited by thyroxin injection. No degenerating and vacuolated forms were present; the relative number was within normal limits. The outstanding difference, however, was in size, which was significantly smaller than that of the basophils in the normal pituitaries (257 ± 7 versus 302 ± 6). The impression was gained that a new crop of basophils was replacing those lost by degeneration.

Injection of growth hormone concurrently with thyroxin had almost the same effect on the pituitary parenchyma as thyroxin alone. The pituitaries were brought almost to nor-

mal under the influence of this combined treatment (fig. 4). The pituitaries differed in only one respect from those of the thyroxin treated group, namely, the regranulation of acidophils was not as complete. They were smaller and fewer in number. The basophils were the same in general appearance, size and number in rats receiving the thyroxin with growth hormone as in thyroxin alone.

No difference between pituitaries of males and females was found which could be ascribed to thyroidectomy or to therapy.

TABLE 3

Percentage of different cell types in pituitaries of rats thyro-parathyroidectomized at birth when treated with growth hormone and/or thyroxin

TREATMENT ¹	NO. OF RATS	Acidophils		Basophils		CHROMOPHOBES	
		Mean	SE ²	Mean	SE	Mean	SE
Thyroxin	6	36.3 ± 1.4		5.5 ± 0.6		58.2 ± 1.4	
Thyroxin and growth hormone	5	28.1 ± 1.6		5.9 ± 0.2		66.0 ± 1.2	
61 to 63 days old, normal controls	6	33.8 ± 1.2		5.2 ± 0.5		61.0 ± 1.5	

¹ Data for untreated, thyroidless rats is not included, due to the diffused outlines or fragmentation of basophils and due to the extremely small numbers of identifiable acidophils.

$$^2 \text{SE (of mean)} = \frac{\text{SD}}{\sqrt{n-1}}$$

DISCUSSION

According to many reports in the literature, the weight of the pituitary is increased by thyroid ablation from young or adult rats. The glands of rats deprived of their thyroids on the day of birth, as described here, were on the contrary invariably lighter. Furthermore, the thyroxin therapy, although resulting in remarkable morphological reconstruction of the parenchyma, did not bring the weight of the pituitaries to normal. It is suggested that the extensive degeneration which had occurred before therapy was instituted may have led to impairment during the very early period of development which was not completely corrected.

The administration of growth hormone to the thyro-parathyroidectomized rats did not improve the pituitary; on the

contrary, the size of the acidophils was reduced and they were further degranulated. Therefore the doubling of body weight found in the growth hormone injected rats (Scow *et al.*, '49) probably should be attributed only to the effect of the injected growth hormone. The depressing effect of growth hormone on acidophils was also noted when it was injected concurrently with thyroxin. In this case, the reappearing acidophils were smaller in size, were less numerous and not so heavily granulated when compared with the same cells in the pituitaries of animals receiving injections of thyroxin alone. A depression of acidophils by growth hormone is in accord with previous studies in this laboratory in which it was found that growth hormone chronically injected into normal, mature rats, and leading to gigantism, reduced acidophil size and granulation (Koneff *et al.*, '48).

In the recent communication of Scow *et al.* ('49), the authors suggested that the dose of thyroxin used restored the production and release of growth hormone by the anterior pituitary. The findings in the pituitary are in accord with this interpretation, since the regranulation of acidophils invariably was demonstrated in thyroxin treated, thyro-parathyroidectomized animals.

Severinghaus ('42) stated that in the thyro-parathyroidectomized rats of Salmon's series, in which thyroid feeding was delayed until the 40th day, there was no restoration of the pituitary in rats in which thyroidectomy was complete; the pituitaries continued to be composed of chromophobes. The present studies were conducted on rats carefully selected as completely thyroidless, yet there were pronounced beneficial effects from thyroxin treatment, judged both by body growth and differentiation and by the restoration of the pituitary parenchyma to the state approaching that of normal controls.

The presence of some deviation from normal found in the pituitary parenchyma of thyro-parathyroidectomized rats treated with thyroxin should not, it seems, be attributed to insufficient dose of the hormone, since even a slight degree of thyroxin deficiency, as was shown by Griesbach and Pervis

('45), would cause increase in number and size of pituitary basophils. Some irreparable damage or definite slowing of development of the pituitary may have occurred during the postoperative period. Attention may be called to the fact that neither body size or differentiation, nor the repair of the endocrine and reproductive organs was reported to be complete under the particular time and dosage conditions of this experiment (Scow *et al.*, '49). With this the pituitary findings agree.

CONCLUSIONS

Pituitary weights of rats thyro-parathyroidectomized at birth were considerably smaller than in normal controls of the same chronological age. Neither thyroxin given alone or growth hormone given alone, nor the combination restored pituitary weight to normal.

Histologically, the pituitaries of thyro-parathyroidectomized, uninjected rats showed extensive degranulation of acidophils. Many basophils displayed multiple vacuolation or degeneration.

Injection of growth hormone did not alter in any respect the basophils, but resulted in further decrease in size and granular content of acidophils.

Thyroxin therapy, alone or combined with growth hormone, restored the pituitary parenchyma almost to the normal condition. The number, size and degree of granulation of acidophils, was, however, somewhat smaller when growth hormone was injected with the thyroxin.

It is suggested that the restoration of the acidophils was responsible for the release of endogenous growth hormone when thyroxin was employed.

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PLATE 1

EXPLANATION OF FIGURES

Mallory-Azan stain. Photographs were taken with 4 mm objective, magnification $\times 400$ (reduced 1/6).

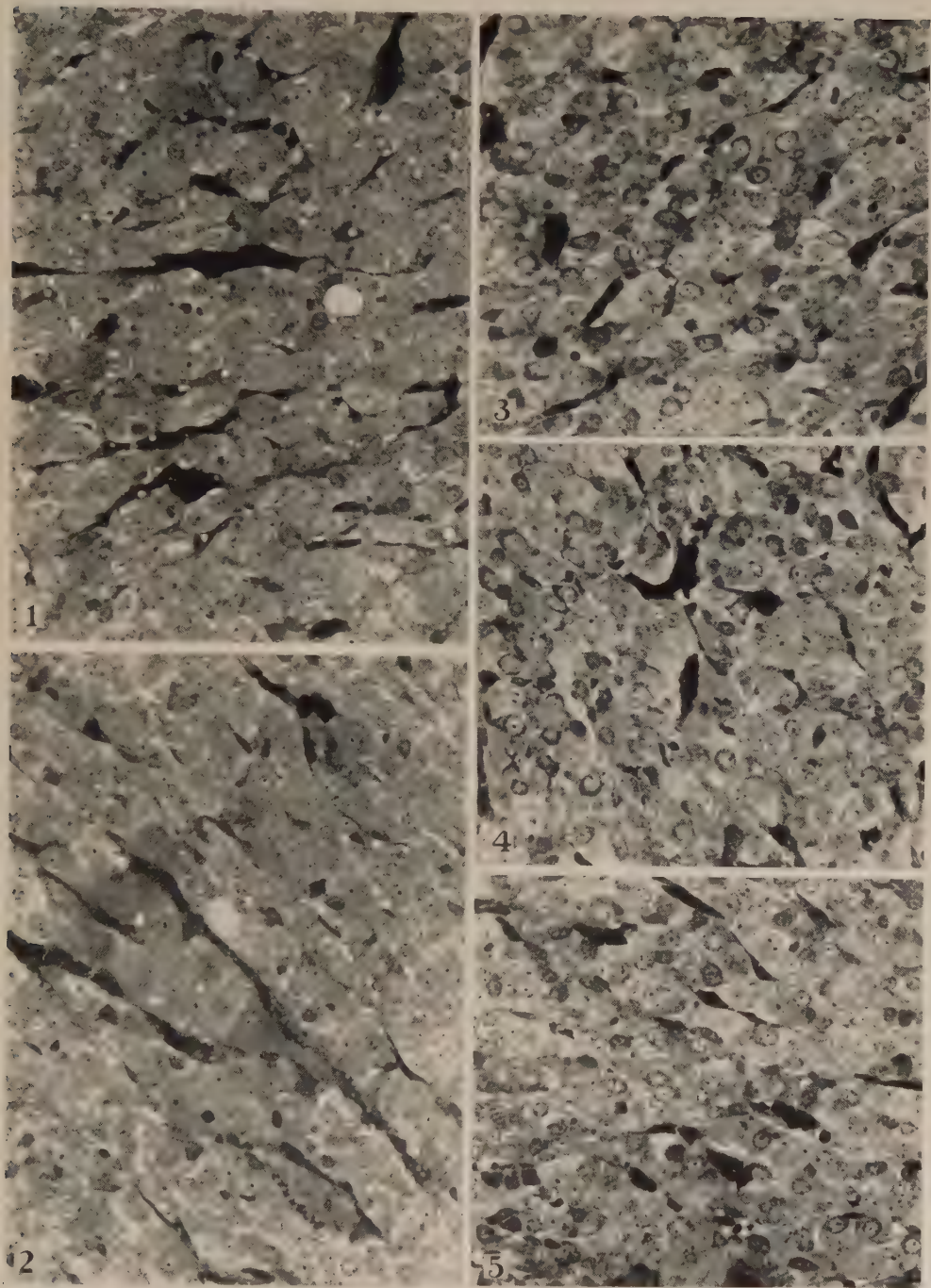
1 Field selected from a section of the anterior pituitary of a 61-day-old female rat, thyro-parathyroidectomized on the first day of life. Note absence of normal acidophils and numerous thyroidectory basophils showing vacuolation and destruction of the cytoplasm. Compare with figure 5 — normal control rat.

2 Field selected from a section of the anterior pituitary of a female rat, thyro-parathyroidectomized on the first day of life and then injected with growth hormone. The injections were begun on the 31st day of life and were continued for 30 days. Note striking similarity with the previous figure.

3 Field selected from the section of the anterior pituitary of a 61-day-old female rat thyro-parathyroidectomized on the first day of life and treated, beginning with 31st day of life, with thyroxin. Compare with figure 4.

4 Field selected from the section of the anterior pituitary of a 61-day-old female rat thyro-parathyroidectomized on the first day of life and treated, beginning with the 31st day of life, with a combination of thyroxin and pure growth hormone for a period of 30 days. Note that the cytoplasm of acidophil cells in both pituitaries (fig. 3 and fig. 4) contain typical granular material. Also note a general resemblance of the structure of the pituitary parenchyma (fig. 3 and fig. 4) to that seen in a normal rat at the same age (fig. 5).

5 Field selected from a section of the anterior pituitary of a normal, female rat of 61 days of age to show general appearance and distribution of cells.



CECAL VILLI IN THE RED TREE MOUSE, *PHENACOMYS LONGICAUDUS*¹

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Department of Zoology, University of California, Berkeley

SEVEN FIGURES

1

The confinement of villi to the mucosa of the small intestine in mammals is generally assumed (Kendall, '47). An exception to this generalization: the presence of similar structures in the cecum of certain rodents, is reported herein.

On March 31, 1949, 8 red tree mice *Phenacomys longicaudus* True, were caught by Dr. Seth B. Benson and Mr. Carl Koford of the California Museum of Vertebrate Zoology near Stewart's Point, Sonoma County, California. During the course of examination for parasites, the ceca were opened and the food masses washed out. Numerous thread-like filaments, projecting from and continuous with the cecal wall, were noted (fig. 2). Under the dissecting microscope a blood vessel could be seen in each filament.

A total of 10 mice, juveniles and adults, have been examined to date, and cecal villi were found in all. Some ceca were rinsed in tap water to eliminate food materials before fixation, and portions of other ceca including food masses were fixed directly in Bouin's fluid, 10% formalin or chilled absolute acetone. Whole mounts of villi were stained with Lillie's modification of Mayer's acid hemalum and with Grenacher's borax carmine. Paraffin sections cut at 6 μ were stained with alum hematoxylin and eosin. The acetone-fixed tissues were used for the histochemical demonstration of al-

¹Aided by a research grant from the University of California Board of Research.

kaline phosphatase by Barger's modification of the Gomori technic, as employed by Bern ('49). These last cecal sections were compared with similarly treated sections of ileum and colon.

ANATOMIC AND HISTOLOGIC DESCRIPTION

The cecum of *Phenacomys longicaudus* is comparable to that of its close relative, *Microtus californicus*, the California meadow mouse, measuring about 65 mm in length and 10–12 mm in width in the adult. It differs from the latter in the



Fig. 1 Diagram of cecum of red tree mouse. Stippled area indicates distribution of villi; a.c., ascending colon; l.p., lymphoid patch; p.s., postcecal spiral; s.i., small intestine. $\times 4/3$.

presence of a postcecal spiral composed of three to 4 closely applied coils (figs. 1 and 2). From the inside of the cecal wall of *Phenacomys* project long villus-like filaments, measuring as much as 11 mm in length in fresh material (fig. 2). Figure 1 illustrates the distribution of these villi, which are present not only in the cecum proper but also in the enlarged portion between the ileocecal junction and the postcecal spiral, extending into the first coil of the latter. Kostanecki ('26) described this enlarged area as the *ampulla caecalis coli* in a series of rodents and claimed it to be structurally distinct from the colon proper, with a mucosa like that of the remainder of the cecum. An ovoid lymphoid patch, meas-

uring about 6×2.5 mm, occupies a constant position halfway between the tip of the cecum and the ileocecal junction, and the mucosa here is generally devoid of villi (fig. 1).

The number of filaments per unit area of cecal wall in *Phenacomys* varied from specimen to specimen. In one subadult male, there were approximately 59 filaments per cm^2 , with a length range of 2.09–8.88 mm, averaging 4.9 mm, and a width range of 0.26–0.56 mm, averaging 0.37 mm. In an adult female, there were about 120 filaments per cm^2 , with a length range of 1.71–6.64 mm, averaging 4.16 mm, and a width range of 0.16–0.41 mm, averaging 0.25 mm. The measurements were obtained from fixed material.¹ Since each filament has a shape approximating that of a cylinder, the average amount of surface presented by the filaments in a given area can be roughly computed, using the average width measurement as the diameter of the cylinder and the average length as its height.

The figure thus obtained for the female discussed above was multiplied by 120 (the average number of filaments per cm^2) to give an average filament surface area of about 4 cm^2 per square cubic centimeter of cecal wall. Additional increase in surface is obtained by cleft-like transverse folds in the surface of the filaments. The surface of each villus also shows numerous pores opening into small unbranched glandular acini similar to the mucosal glands in the wall of the cecum (figs. 4–6).

The surface epithelium of the cecal villi is similar to that of the cecal wall, consisting of tall columnar, eosinophilic cells with a distinct thick cuticula. The cells of the acinar glands are largely basophilic and connect with the surface epithelium by means of short ducts with narrow lumina (fig. 6). The entire surface epithelium is intensely positive for alkaline phosphatase activity, particularly in the apical regions of the cells, whereas the acinar cells are largely negative (fig. 7).

Internally (fig. 5), the cecal villi contain an arteriole, one or more venules, lymphatic spaces, lymphoid tissue, very little fibrous connective tissue, and a small number of smooth

muscle fibers arranged parallel to the longitudinal axis of the villus. A rich subepithelial capillary plexus is also evident.

The cecal villi, although possessing a phosphatase-positive epithelium like those of the ileum, differ from the latter (fig. 3) in their larger size, elaborate internal structure, glandular inpocketings, absence of goblet cells, and considerably taller epithelium. The phosphatase-positive epithelium extends into the postcecal spiral beyond the villous area; however, the posterior end of the spiral, like the ascending colon, shows little phosphatase activity in its surface epithelium.

The position of the filaments in a cecum filled with food is noteworthy in that they are imbedded in the food mass with their tips extending toward the center of the lumen. Their length and number thus enable them to reach the food mass from all sides.

DISCUSSION

The postcecal spiral described by Tullberg (1899) for *Lemmus* closely resembles that of *Phenacomys*. Patzelt ('25) described a double spiral of the *colon ascendens* in *Microtus (Arvicola) terrestris*, which, however, differs from those found in *Lemmus* or *Phenacomys* in that the coils are apparently not closely applied to each other.

In none of the rodents previously discussed in the literature have cecal villi been demonstrated. Structurally and functionally these filaments in *Phenacomys* basically resemble villi of the small intestine, and the absorptive surface of the cecum is greatly increased by their presence. It is also of interest that the cecal epithelium, like that of the ileum, shows strong alkaline phosphatase activity, inasmuch as this activity is often correlated with carbohydrate transport mechanisms (e.g., Willmer, '44). It is possible that the villi may impede somewhat the passage of food through the cecum.

Since *Phenacomys longicaudus* feeds primarily on needles of Douglas fir (*Pseudotsuga taxifolia*) and grand fir (*Abies grandis*), it was thought that the structure of the cecum might

be an adaptation to the specialized food habits of this mouse. A considerable quantity of needles is consumed each day (Howell, '26; Benson and Borell, '31). To examine this idea of adaptation to specialized food habits, the cecum of another species, *Phenacomys intermedius celsus*, was examined. This mouse lives at high altitudes and apparently feeds on heather (Howell, '26). Its cecum, however, was quite similar to that of *P. longicaudus*, as was that of *P. albipes*. Subsequently, the following additional members of the subfamily Microtinae were examined: *Microtus californicus*, *Clethrionomys californicus*, *C. gapperi*, *Lagurus curtatus*, *Lemmus nigripes*, and a very young specimen of *Ondatra zibethica*. In none of these were cecal villi found.

Mangold ('29) stated that the villi in the rumen of cattle may be up to 1 cm long, and those in domestic sheep up to 5 mm long. As far as the authors have been able to determine, these are the only mammals possessing villi of lengths comparable to those of *Phenacomys* cecal villi. Tullberg (1899) in describing the digestive tract of the Siberian lagomorph, *Lagomys alpinus* (*Ochotona alpina*?) mentioned the presence of numerous long, branched villi on the rim of the spiral valve of the cecum, which he considered to be projections of the mucosa. He did not give any information as to the number, size or structure of these villi. Examination of an alcohol-preserved specimen of *Ochotona princeps* demonstrated structures similar to those mentioned by Tullberg. In *O. princeps*, these "villi" are present on the rim of the spiral valve only, and, in contrast to *Phenacomys*, are thus restricted to a relatively small part of the cecal wall. In addition, they are shorter, wider and flatter than those of *Phenacomys*.

SUMMARY

Cecal villi are described from the red tree mouse, *Phenacomys longicaudus*. The villi are present in the cecum proper and in the *ampulla caecalis coli*, extending into the first coil of the postcecal spiral. Similarities and differences between the ileal and cecal villi of *Phenacomys* are recorded, includ-

ing intense alkaline phosphatase activity in the epithelium of both. The long cecal villi (up to 11 mm in length), unlike the intestinal villi, are equipped with numerous acinar glands.

No villi were found in the cecum of 5 other genera of the subfamily Microtinae to which *Phenacomys* belongs.

ACKNOWLEDGMENTS

We wish to thank Dr. Seth B. Benson for the loan of alcohol-preserved specimens and Professor John Gullberg for the photograph included herein as figure 2.

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PLATE

PLATE 1

EXPLANATION OF FIGURES

2 Cecum of *Phenacomys*, opened and washed free of food mass, showing villi; three coils of postcecal spiral at right. Note villi extending into first coil of postcecal spiral. $\times 1$.

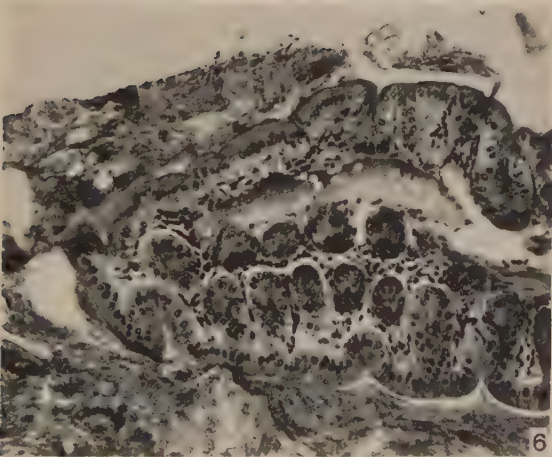
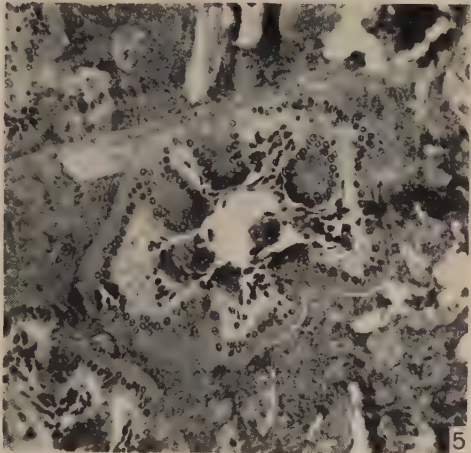
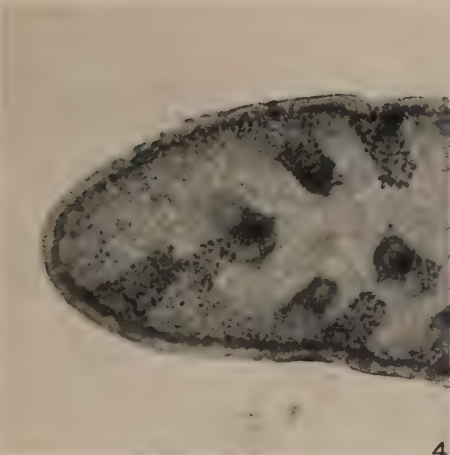
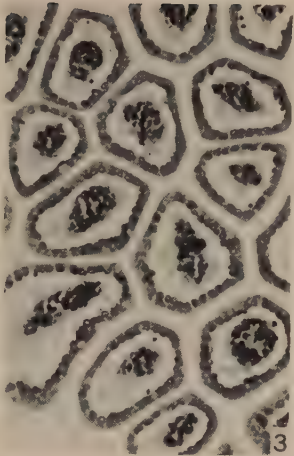
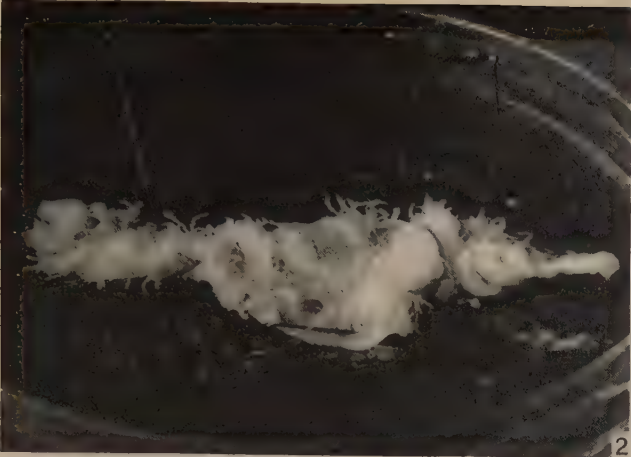
3 Cross section through villi from wall of ileum. H and E. $\times 140$.

4 Tip of whole mount of cecal villus, fixed in formalin and stained with acid hemalum. Note acinar glands. Lymphoid tissue is absent from this region. $\times 140$.

5 Cross section through cecal villus imbedded in food mass. Note glandular impocketings, blood vessels, lymph spaces, tall columnar epithelium. Cf. fig. 3. H. and E. $\times 140$.

6 Longitudinal section through portion of cecal villus to show basophilic acinar glands. H. and E. $\times 140$.

7 Longitudinal section through portion of cecal villus to show intense alkaline phosphatase activity of surface epithelium and virtually negative acinar epithelium. Acetone-fixed tissue prepared according to Barger-Gomori technic; nuclear coloration due to hematoxylin. $\times 140$.



HUMAN EPIDERMAL CELLS OBSERVED IN TISSUE CULTURE WITH PHASE-CONTRAST MICROSCOPY ¹

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NINETEEN FIGURES

Living cultures of human epidermal cells offer a challenging opportunity for the study of cellular activity in relation to chemical agents and immune mechanisms. The effect of antiseptics, chemotherapeutic substances, so-called epithelializing agents such as scarlet red, the chlorophyll compounds, ultraviolet light at a wave length of less than 3200 Å, and radio-active elements on cytoplasmic, mitochondrial and chromosomal movement, may be analyzed from photographic records. The importance of such studies in relation to problems in the reparative procedures of plastic surgery notably in the enigma of homoioplastic skin grafting, the induction of malignancy by physical and chemical agents, and the special reaction exhibited in the histopathology of skin diseases, led us to undertake the development of a simplified *in vitro* method which would permit rapid direct observations on the living elements of the human skin.

MATERIALS AND METHODS

A record form calling for relevant data on the patient and the conditions under which the skin was obtained was designed so that when sufficient information was obtained correlations might be established between the outgrowth of various cells

¹ Aided by a Plastic Surgery Special Activities Fund administered by T. G. Blocker, Jr. and a grant from the American Cancer Society, CP 12 A.

in relation to such variables as age, skin color, area of the body, antiseptic preparation, novocaine infiltration, blood chemical factors, the relative thickness of the explant and other related data.

A simple method of obtaining biopsies consists of applying the jaws of a rubber protected, intestinal clamp to the thin, loose skin of the extremities which permits partial analgesia to develop after a few minutes of ischemia, and then shaving slips of tissue with a wafer razor blade. Strips of skin approximately $\frac{1}{4}$ " in width obtained parallel to surgical incisions are particularly useful for studies involving the deeper zones of the dermis. The bulk of our observations, however, were based upon cultures of explants prepared from remnants of split grafts used for reparative procedure. These are especially useful in that they consist of presumably healthy skin, their thickness is relatively uniform and they are handled with a minimum of trauma.

Explants approximately 1.5 mm square were prepared with the precaution that all cutting was done with very sharp knives so as to avoid the production of jagged margins. Fragments of skin were washed with Tyrode's solution. The addition of a small amount of human or chick serum to the Tyrode is believed to enhance its physiological properties. Work in progress involves the use of roller tube, Carrel flask and Gey's ('48) thin metal plate phase technique but the majority of observation reported in this paper were based on maximum lying-drop or simple hanging drop culture technique.

For such preparations one drop of cockrel plasma was deposited on a sterile $\frac{3}{8}$ " square cover glass to which a fragment of skin was transferred. After the addition of one drop of embryonic extract obtained from 7-day chick embryos the preparation was mixed thoroughly and allowed to clot. One to two drops of human ascitic fluid was then added to the clotted surface and a hanging drop slide was affixed in the usual way. Cultures were generally incubated at 37°C. Results obtained in relation to lower temperature will be reported in a later communication. Grateful acknowledgement is made

to Drs. T. G. Blocker, Jr., and J. H. Hendrix for continued interest and cooperation in this study.

OBSERVATIONS

Outwandering of epithelial cells from explanted skin was seen in as little as 18 hours. This generally consisted of a compact sheet although occasionally large isolated epithelial elements were found. Cinematographic records gave proof that such cells were capable of division. Evidence is being accumulated to show that a clone may arise from individual wandering epithelial cells. The practical significance of this phenomenon in the repair of denuded skin is obvious but unquestionably requires ideal conditions for success. Isolated cells have also been observed to return to the outwandering epithelial sheet and to become reincorporated in it. Figures 1 and 2 taken on the 4th and 5th day respectively indicate the change in the amount of outgrowth during a 24 hour period. This skin was obtained by the wafer-blade skin clamp method and did not involve the use of ascitic fluid in its preparation. More typical of the results which were obtained with the use of split grafts and with the addition of ascitic fluid to the clotted medium, is the preparation shown in figure 3. Under these conditions epithelial sheets, usually encircling explants and generally consisting of 20 to 30 cells in depth, could be obtained with regularity.

Microcinematographic records of activities in living cultures are being made with the use of the ordinary light microscope and with a Spencer phase-contrast optical system. Time-lapse photography at rates varying from one to 8 frames per minute is achieved with the use of a Rolab timer.

Successful cultures have been obtained from the skin of more than 70 human subjects. The present report is concerned with illustrating characteristic results and, therefore, figures 4 to 19 were all made of one culture from the skin of patient T. J. JSH no. 7240 using a 1.9 mm Spencer dark-phase objective and a 10 × Zeiss apochromatic ocular at an initial magnification of 2000 × using bellows extension. The

preparation was made from a split graft taken from the left epigastric area which had been prepared by cleansing with soap, water, ether, $\frac{1}{2}\%$ iodine in 95% alcohol followed by immediate rinsing with ether. The patient was a 27-year old colored male. The grafting procedure being used for the repair after excision of a sarcoma in an old healed burned scar of the lateral surface of the right thigh.

The phase preparations were made in the following manner. A 40×60 mm no. 1 cover glass was affixed to one side of Gey's thin metal ring slide with vaseline. A thin circle of paraffin approximately 1 cm in diameter was applied on the "well side" of the cover glass and a small drop of ascitic fluid was placed on the center of the glass. The $\frac{3}{8}$ " square cover glass containing the out-growing skin culture was then transferred to the phase preparation so that the clotted medium made uniform contact with the fresh ascitic fluid. Finally, the margins of the square glass were sealed with paraffin. Such preparations are generally allowed to equilibrate to the special conditions occasioned by transfer to the new situation from two to 6 hours before photographic records are made. The activity of the membrane as seen in figures 8 and 9 and the appearance of the mitochondria shown in figures 10 and 11 suggest that relatively favorable conditions are maintained following the procedure which has been described.

Indications of polarity can be seen in the asymmetrical arrangement of cytoplasmic structures in figures 4 to 7. These illustrations also show that the nuclear membrane is sharply defined and smooth. The nucleoplasm is not markedly dense. Light spots seen in the nuclei in figures 5 and 7 represent granulations in a somewhat more superficial plane of focus. Nucleoli are notably variable in number, size and shape (figures 4 to 7). Masses near the nuclei shown in figures 6 and 7 are believed to be debris consisting chiefly of melanin granules.

Human epidermal cells migrating in tissue cultures showed a high degree of mobility at their margins (figs. 8 to 15). Most characteristic of this activity were the relatively broad

waves of an undulating membrane as seen in figures 8 to 12. Cinematographic records established that in some areas the marginal movements involved the formation of conspicuous vacuoles by the process described by Lewis ('31) as pinocytosis. Dark lines seen in the area between the active margin and the zone containing collections of vacuoles as seen in figure 9 were very conspicuous in some cells observed at a particular focal plane. These could be shown to represent the surface view of the undulating membrane rather than mitochondrial filaments. Figures 10, 11 and 16 show the long filamentous mitochondria typical of the epithelial cells in our cultures. Mitochondria were often present in close association with the vacuoles of an area of intense pinocytosis.

Figures 12 to 15 represent a variety of special formations seen at the margins of healthy cells whose interpretation has not yet been fully clarified by cinematographic analysis. The pattern shown in figure 12 probably represents the result of cytoplasmic movement in the direction of the wavy margin with the production of filamentous strands on the opposite side as a consequence of tensions on adherent cytoplasm. Such filar structures, however, are very common in cultures of epidermis (figs. 15 and 16) and may be related to the "intercellular bridges" seen in histological sections of the skin.

Contact between epithelial cells is illustrated by figures 16 to 19. Figure 16 shows the presence of mitochondria intimately associated with the zone of contact between two cells. At a more critical level of focus the characteristic picture of cell boundaries is seen to resemble the pattern of a "greenstick" fracture of bone (fig. 5, 17 and 18). Arrows in figure 18 indicate boundaries between the cell occupying most of the field and 4 other elements. Some evidence was found for a cytoplasmic fibrillar system which might extend between two cells. The pattern is indicated by an arrow in figure 17. These may be tonofibrillae as seen with dark-phase contrast microscopy. Strong fibrillar systems were found in some areas of the colony which may have indicated special conditions of mechanical stress (fig. 19).

Using phase microscopy Gey, Gey, Firor and Self ('48) observed clearly defined fibrillae in thinly spread cytoplasm of rat fibroblasts. The observations were extended by Bang and Gey ('48) in preparations using the electron microscope. These authors signal the importance of further studies in this direction especially in the light of current concepts of the basic fibrillar structure of cytoplasm (cf., Frey-Wyssing, '48; Monné, '48).

DISCUSSION

While procedures for the growth of epidermis have been used for many years by investigators utilizing tissue culture technique, recent experimental studies on human skin have stimulated new interest in this field. Thus, Doljanski, Hoffman and Tenenbaum ('42) concluded that extracts of heterologous adult tissues stimulated human skin cultures to a notable degree and that such extracts might prove effective in stimulating wound healing. A growth promoting principle was described in a partially purified heart muscle extract by Werner and Doljanski in 1942.

A physiological solution free of calcium, containing 9mM/L of phosphate, and with a pH of 6.9 was found to favor the growth of epidermis by Parshley and Simms ('46). These investigators also found that neutralized aspartic acid was highly stimulating to epithelium *in vitro*. The use of a thick plasma clot was recommended since epithelial cells digest fibrin rapidly and this seems essential to good growth.

In a study concerning the growth of normal and malignant cells incubated at temperatures below 37°C., as well as under conditions of minimal feeding, Gey, Hanks and Barrett ('48) reported that pure epithelial cell growths from the skin were maintained at 31°C. for nearly 6 months before being discarded. It was not found possible, however, to carry such cells in continuous culture by the method of serial transfer.

The present study indicates that ascitic fluid serves as an excellent component in the medium utilized in a simple culture preparation especially adapted for short term studies. Phase-contrast microscopy has proved to be a valuable aid for di-

rect studies upon the cytological components, particularly the mitochondria, of human epidermal cells. This is illustrated by photomicrographs made on various cells from a single explant.

SUMMARY

1. Human epidermis has been successfully cultivated *in vitro* in a medium consisting of one drop of cockrel plasma and one drop of embryonic extract obtained from 7-day chick embryos. One to two drops of human ascitic fluid added to the clot has been found to enhance the growth of the epithelial cells.

2. Simple hanging drop preparations were transferred to mounts of suitable thickness for photomicrography and cinematography using a 1.9 mm Spencer dark-phase contrast objective.

3. Sharply defined nuclear membranes, activities at the edge of the membrane including pinocytosis, mitochondria and intercellular structures are described and illustrated.

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PLATE 1

EXPLANATION OF FIGURES

Figures 1-19 Human epidermal cells in tissue culture. All photographs were taken of the living preparations. Figures 1-3 were made with the light microscope. Figures 4-19 represent dark phase-contrast photomicrographs taken with a 1.9 mm Spencer objective and a 10 $\times$ Zeiss ocular. The magnification of all phase illustration is approximately 1500 $\times$.

Figure 1 shows outgrowth from a "wafer-blade" biopsy cultured without the addition of ascitic fluid after 4 days of incubation at approximately 45 $\times$.

Figure 2 is the same preparation as that shown in figure 1 but photographed 24 hours later.

Figure 3 Outgrowth of epithelium from epidermis obtained from split-graft cultivated in a hanging drop preparation in which two drops of ascitic fluid had been added to the clot. Seventh day of incubation. Explant is represented by the dense material on the right. Magnified approximately 135 $\times$.

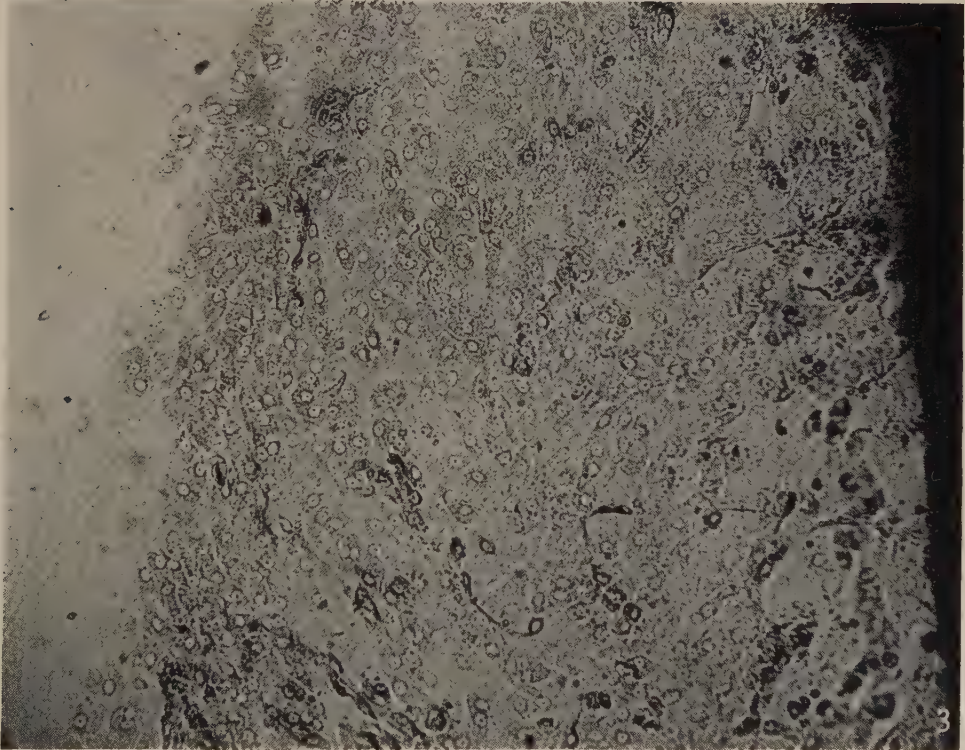


PLATE 2

EXPLANATION OF FIGURES

Figures 4-7 Central portion of epithelial cells showing the structure of nucleus and adjacent areas. Pigmentary debris is seen in figures 6 and 7.

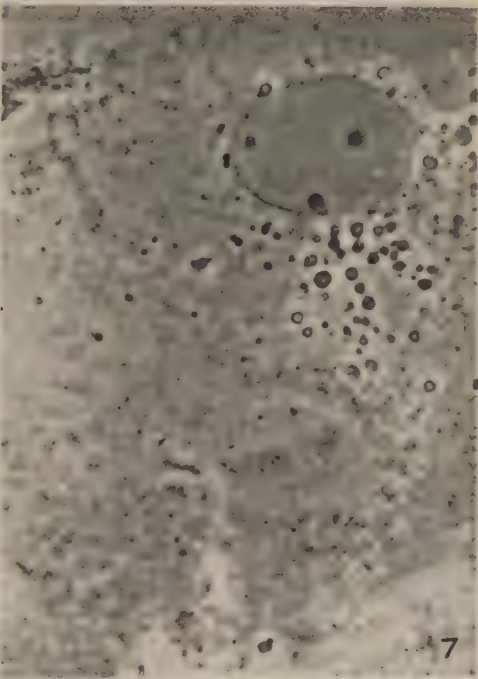
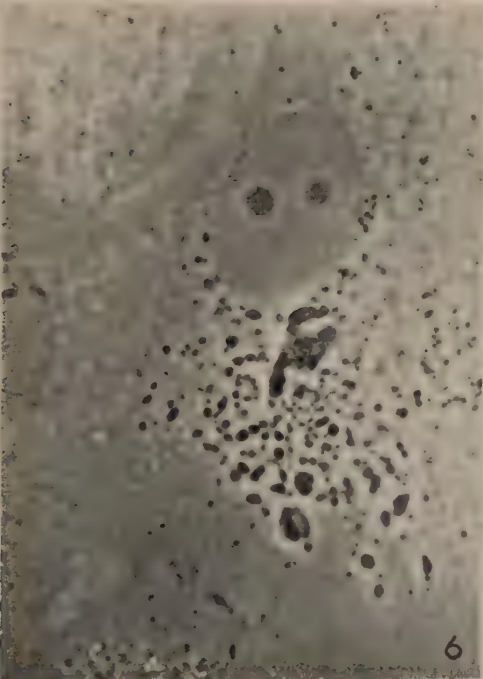
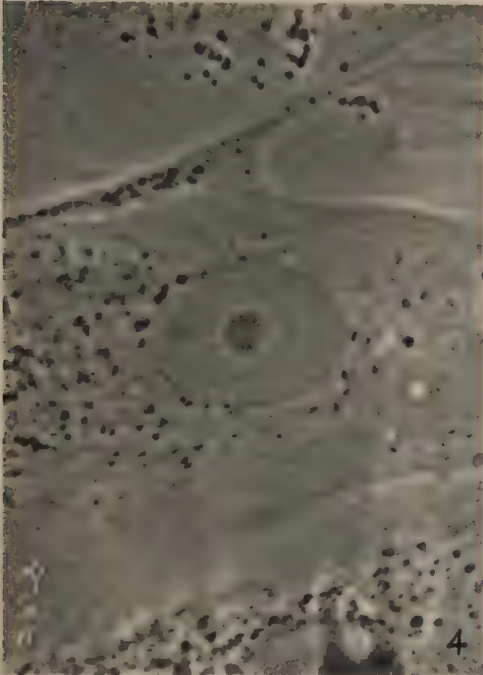


PLATE 3

EXPLANATION OF FIGURES

Figures 8-11 Margins of epidermal cells showing various patterns commonly seen in the undulating membrane. Vacuolar formation (pinocytosis) is conspicuous in figures 9 and 11. Characteristic filamentous mitochondria are seen in figures 10 and 11.

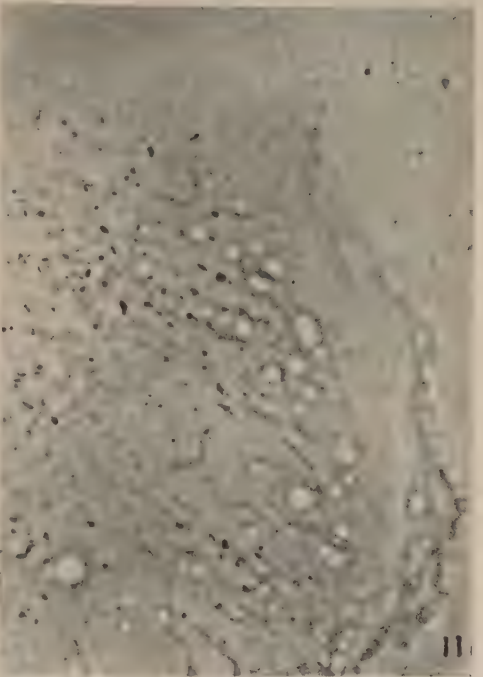
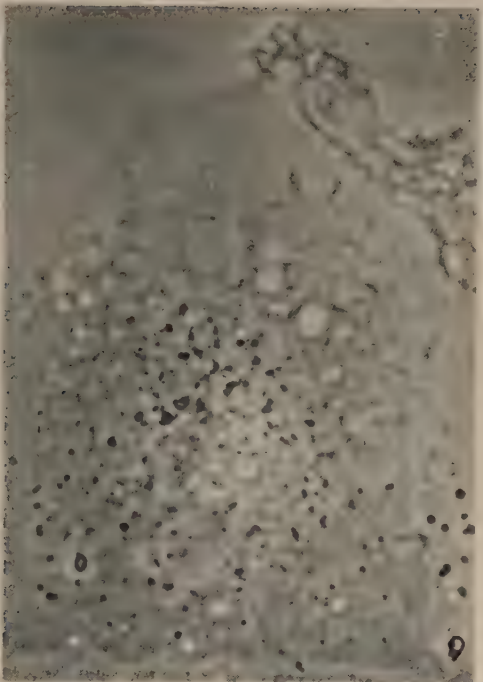
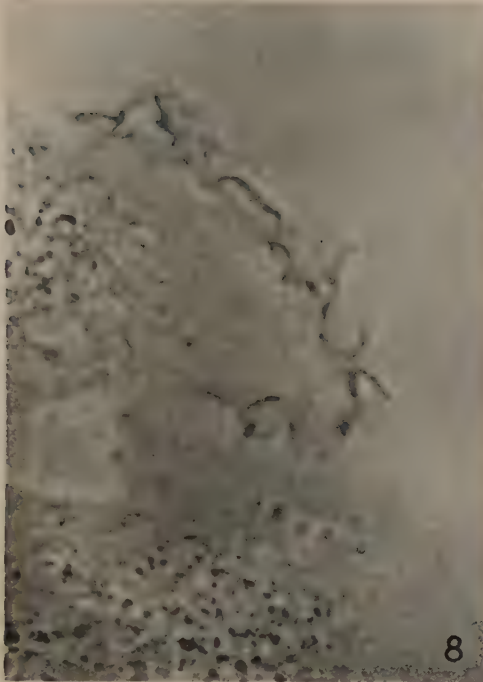


PLATE 4

EXPLANATION OF FIGURES

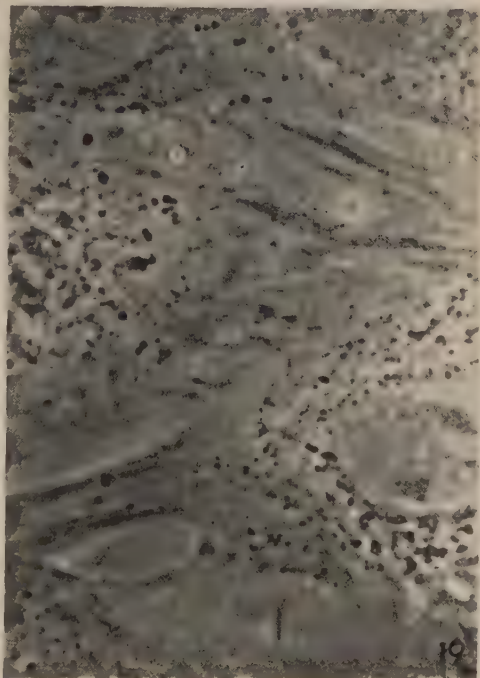
Figures 12-15 Special patterns occasionally found at the margins of epithelial cells. The arrangement seen in figure 12 probably resulted from the movement of cytoplasm in the direction of the undulating membrane. The filamentous processes on the opposite side resulting from the tension exerted on the cytoplasm adhering to the medium or to the cover glass.



PLATE 5

EXPLANATION OF FIGURES

Figures 16-19 show relations between epithelial cells, figure showing mitochondria concentrated near the boundary of the two cells. Arrows in figure 18 indicate points of contact between cells. The arrow in figure 17 is directed toward an intra-cellular fibrillar system. Figure 19 shows strong tension lines between cells in a zone of cells close to the explant.



BOOK REVIEW

EVOLUTION OF THE FOREBRAIN. By G. W. H. SCHEPERS, MASKEW MILLER, LTD., CAPETOWN. 212 pp. 1948.

The introductory chapter of this book impresses the reader with the soaring scope of the work. The author is fascinated by the evolution of the telencephalon from a more or less undistinguished portion of the brain to the remarkable center of intellection that it has become in man. The tracing of the evolutionary phases which have brought this about is a task of almost incredible proportions. It appears that the aim of this work, rather than its content, must be called upon to justify its expansive title. But, despite the brevity of the treatment, much original material is contributed and stimulating discussions of neuro-evolution are included.

Chapters II to XI describe the telencephalon of *Testudo geometrica*, the South African land tortoise, and compare it with the telencephala of other reptilian forms. Concise tables of reptilian telencephalic homologies are included here. These chapters are thorough and tidy, forming a contribution to descriptive neurology which reflects great credit on the author. In addition to the routine descriptions of surface conformation, cell masses and fiber tracts there is a good account of the vascularization. The illustrations, with the exception of some in chapters III and IV, are excellent. This section of the book is a commendable treatment of its subject.

Chapters XII to XVI are devoted to the comparison of the telencephalon of *Testudo geometrica* with those of other forms, from vertebrate to primate. It is more interpretive than descriptive. The effort to synthesize the mammalian telencephalon from its simpler homologies in lower forms is clear, but is replete with highly debatable interpretations. A multitude of previously published theories, dealing with subjects such as the pre-vertebrate origins of the telencephalon, etc., are described and discussed. The author is critical and opinionated but writes clearly in a stimulating fashion. This section should be interesting to all but will be agreed with in its entirety by no one. Comparative neurologists fond of argumentative discussions are advised that the closing chapters of this book are an excellent springboard for the exercise of that activity.

The book is well documented throughout although, in the more general discussions, all authors are not cited with reference to specific works. Dr. Schepers is obviously well read in his field and keenly interested in the basic questions therein. There is no index, but certain of the chapters are preceded by an outline of the material to be covered. As a descriptive account of the telencephalon of *Tes-tudo geometrica* this work is highly recommended. The more controversial chapters can be read profitably for the vast amount of neurological theory résuméd in them, but their controversial nature should remain foremost in the mind of the reader.

F. N. Low

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